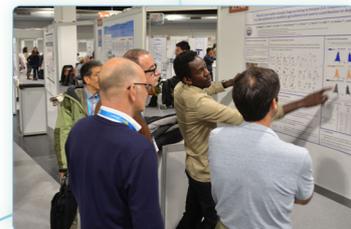**Joint FAO/IAEA Programme**
Nuclear Techniques in Food and Agriculture

# FAO/IAEA
# Agriculture & Biotechnology
# Laboratories

*Activities Report* **2017**

**IAEA Laboratories Seibersdorf, International Atomic Energy Agency, Vienna, Austria**

# CONTENTS

# THE ANIMAL PRODUCTION AND HEALTH LABORATORY

## EXECUTIVE SUMMARY

The Animal Production and Health Laboratory (APHL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture assists Member States improving the productivity of livestock and preventing and controlling animal infectious diseases and zoonoses. Nuclear and nuclear related techniques for serology, molecular biology and genetics are the main technologies applied in the APHL and transferred to Member States' laboratories to support livestock productivity and animal health.

Animal infectious diseases represent one of the main limiting factors to livestock productivity and cause important economic losses. For example, peste des petits ruminants (PPR), a highly contagious viral disease of wild and domestic small ruminants, has spread to more than 70 countries in Asia, Africa and Middle East with an estimated annual loss ranging from US $1.45 billion to $2.1 billion. A study conducted in Nigeria, one of the largest poultry economies in Sub-Saharan Africa, estimated total chicken deaths attributable to Newcastle disease (ND) – a deadly viral disease of poultry - to be approximately 25.5 million heads, with a reduction of 26.5 million units in egg production. The financial burden of ND in Nigeria is estimated in 8.9 billion naira (approximately, US $56 million). Furthermore, several of these infectious diseases can be transmitted from animals to human (zoonoses), causing serious illnesses and public health concerns. In countries with limited resources, the incursions of these diseases also have negative impacts on food security, particularly in the rural communities.

At present, vaccines are primary tools in the prevention and control animal infectious diseases. The APHL is conducting research to develop and select proper vaccine candidates for a new generation of safer, cost-effective animal vaccines by investigating the application of gamma irradiation on pathogens (viruses, bacteria and parasites) and hence to expand the availability of such vaccines. Through past and present coordinated research projects (CRPs), the APHL collaborates with scientists in Member States to develop irradiated vaccines for animals, for example against animal trypanosomas, small ruminant gastro-intestinal parasites (*Haemonchus* spp.), brucellosis, pasteurellosis and avian influenza. In 2017, the APHL conducted studies on the application of irradiation to prepare antigens and inactivate viruses responsible for important diseases affecting swine production, such as porcine reproductive and respiratory syndrome (PRRS) and swine influenza. The application of gamma irradiation has also been investigated to explore the possibility to develop more effective and safer vaccine formulations. In this regard, research activities were developed to study the effects of irradiation on adjuvants incorporated into vaccine formulations to enhance their ability to activate the immune system of vaccinated animals. Liposomes, nanoscale molecules with at least one lipid bi-layer used as carriers to deliver vaccine antigens, are effective adjuvants in several vaccine formulations. An investigation was conducted on the effects of irradiation on the adjuvant properties of liposomes and the possible use of this technology to sterilize these molecules. The effectiveness was assayed through specific immunological test developed at the APHL, such as the antigen uptake ability and maturation of bovine monocyte-derived dendritic cells (MoDC).

The APHL also assisted Member State laboratories by developing and transferring assays facilitating the assessment of vaccine efficacy, with particular focus on the evaluation of the cell mediated immune response to vaccines. The assay panel under development at the APHL includes cytokine expression analysis by quantitative real time PCR (qPCR) with sheep, cattle and goats as the priority. Twenty-five interleukins, and two calibrator genes have so far been optimised using cells stimulated *in vitro* for interleukin production.

To facilitate an effective control of zoonoses and transboundary animal diseases, early detection and rapid confirmation of the identity of the pathogen are essential. The APHL continued to work on the development, validation and transfer of nuclear derived serological and molecular techniques for

disease surveillance, the sensitive and specific detection of pathogens and the application of molecular epidemiological studies for disease tracking and outbreak investigation.

R&D activities at the APHL led to the development and validation of a new rapid test for the sensitive and rapid detection of PPR virus (PPRV) and improved serological assay for the detection of neutralizing antibodies against capripox viruses, including Lumpy Skin Disease. In collaboration with Member States laboratories, genetic sequencing of pathogens was applied to investigate the molecular epidemiology and infer the origins of important transboundary animal diseases, such as African Swine Fever in the African pig population, Newcastle Disease and fowlpox in poultry in Southern Africa, as well as Brucella and Pasteurella in Asia.

Under animal genetics, the APHL completed the construction of two radiation hybrid (RH) panels (5000RAD and 15000RAD) for mapping camel genome. This important genomic resource is now available for use by Member States to construct whole genome camel RH maps and develop genomic tools for camel improvement. The APHL also completed the development and validation of real time PCR based genotyping assay to detect sheep carrying FecB mutation for increased litter size (twins and triplets). The new genotyping tool is rapid and cost-effective and will help Member States to improve the efficiency of marker assisted breeding for improved prolificacy in sheep. During 2017, significant achievements were made in supporting the Member States to implement their national plans of action on animal genetic resources. With the APHL support through IAEA technical cooperation projects, Sudan completed molecular genetic characterization of six cattle breeds, three goat breeds and six sheep breeds while Burkina Faso completed genetic characterization to generate baseline information on six indigenous Guinea Fowl populations.

In addition to R&D, the APHL was also involved in capacity building activities in IAEA and FAO Member States. An online training course on the use of the "Genetics Laboratory Information and Data Management System (GLIDMaS)" was implemented using Webex platform. The training course is expected to help Member States harmonize livestock genetic data and enhance their data management capabilities on livestock biodiversity.

One workshop and two training courses on transboundary animal and zoonotic diseases were organized in Vienna and Seibersdorf during 2017. Two additional training courses on animal and zoonotic disease diagnoses were organized in Ethiopia and Vietnam. The APHL also hosted ten fellows for training on animal disease diagnoses, animal genetic resource characterization, bioinformatics analysis of livestock genetic/genomic data and molecular epidemiology. The APHL staff undertook three technical field support missions in Member State laboratories and institutions (Mongolia, Myanmar and Vietnam) to support activities related to animal health and animal production.

Technical data and scientific information were shared with Member States and scientific communities through 16 publications in refereed high impact scientific journals and two international congresses.

The APHL is part of and continues to coordinate and support the VETLAB Network of national veterinary diagnostic laboratories, in 44 African and 19 Asian countries.

APHL activities, in particular capacity building and technology transfer, also benefited from the financial support from USA and Japan through the IAEA Peaceful Uses Initiative and from South Africa through the African Renaissance Fund.

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[1] Assignment finished in February 2017; [2] Assignment finished in August 2017

# MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

## Animal health

### *Use of radiation technology to develop a new generation of vaccines for animal infectious diseases*

**Irradiated porcine reproductive and respiratory syndrome vaccine yields cell mediated immune responses**

Porcine reproductive and respiratory syndrome (PRRS) is now found in most areas of the world, inflicting major economic impact on the swine industry. Modified live-attenuated vaccines (MLV) against PRRS virus have been widely used, but have failed to provide complete protection against emerging and heterologous strains of the virus (i.e. different from the strain contained in the vaccine preparation). Moreover, there are reports showing MLV reverting to virulent forms and safety of the vaccine is a major concern. Therefore, a killed (inactivated) or a subunit vaccine is much sought after. Irradiation is an attractive approach, in comparison to traditional methods of inactivation, to inactivate viruses and hence to develop vaccines where the protein antigens are relatively well preserved. Against this backdrop, the APHL, in collaboration with the Austrian Agency for Health and Food Safety (AGES) is developing an irradiated PRRS vaccine prototype. The highly pathogenic type 2 PRRS virus (PRRSV) strain Vietnam AGES/568-30FC/13 was irradiated at 30 kGy (irrPRRSV) and its inactivated status, as a measure of safety, was confirmed through cell culture passaging. The irrPRRSV was then used as candidate vaccine and injected intra-muscularly on one-month old piglets. In addition, swine influenza virus H3N2, previously isolated from a pig in Austria, was irradiated at 30 kGy (irrSIV), its inactivated status confirmed through cell culture passaging, and injected separately or in combination with an adjuvant in animal groups. Two vaccine doses were given at two-week intervals followed by a third three weeks after the last vaccination. Animals were then sacrificed and cell mediated immune responses were measured. Peripheral blood mononuclear cells (PBMCs) were obtained from the sacrificed animals and re-stimulated with the inactivated virus. Following overnight incubation, cytokine production was measured in CD4 and CD8 lymphocytes by staining with fluorochrome conjugated antibodies against specific markers and analysed through flow cytometry. Results suggest that there is a substantial cell mediated immune response with vaccination of irradiated PPRSV as indicated by increased percentage of poly-functional (IFN-gamma and TNF-alpha cytokine producing) CD4 and CD8 lymphocytes following irrPRRSV vaccination as compared to irrSIV or a combination of irrPRRSV and irrSIV (Figure 1). These experiments are ongoing.
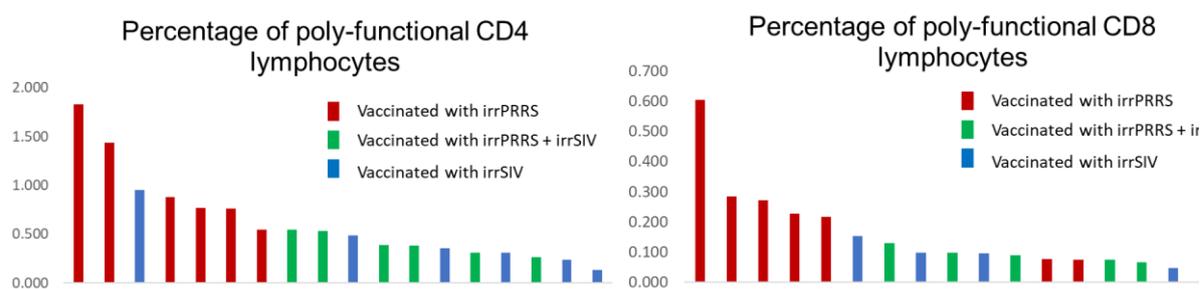


FIG. 1: *Percentage of poly-functional CD4 (left) and CD8 (right) cells in peripheral blood from swine vaccinated with irrPRRSV (red), irrSIV (blue) or a combination of irrPRRSV and irrSIV (green). Each bar represents one vaccinated animal*

**Irradiation of vaccine adjuvant liposomes containing monophosphoryl lipid A preserves ability to augment antigen uptake activity**

Vaccines are a very effective and relative low-cost tool in preventing infectious diseases. In many vaccine preparations, an adjuvant is incorporated to enhance the ability of the vaccine antigen to activate the immune system. Liposomes are nanoscale molecules with at least one lipid bi-layer used

as carriers to deliver vaccine antigens, making them effective adjuvants in vaccine formulations. On the other hand, monophosphoryl lipid A (MPLA) is a compound that is derived from lipopolysaccharides of the cell walls of gram negative bacteria and is used as a safe and extremely potent way of activating the cells of the innate immune system. Therefore, liposomes containing MPLA are excellent vaccine adjuvants and have been used in many vaccine formulations to increase their potency. Since most vaccines are injectables, it is important that they are sterile. Among other methods, filtration is the method of choice for sterilizing liposomes. However, if the liposome is attached to an antigen larger than 0.2 μm, filtration is not possible. Therefore, alternative methods such as irradiation were explored. Moreover, irradiation has also been explored by the APHL to inactivate or attenuate pathogens to develop new vaccines. We therefore investigated the effects of irradiation on MPLA-liposomes and assayed their effectiveness through antigen uptake ability and maturation of bovine monocyte derived dendritic cells (MoDC). This was a collaborative effort with Polymun Scientific Immunbiologische Forschung GmbH in Vienna, Austria. Results indicate that MPLA-liposomes do indeed augment antigen uptake by bovine MoDCs and that the irradiation of MPLA-liposomes at a dose of 25 kGy at room temperature does not change this property. This was confirmed by antigen (dextran) uptake ability (Fig. 2). We have also previously shown that maturation of MoDCs is unaffected when pulsed with irradiated or non-irradiated MPLA-liposomes. Furthermore, the physical and chemical properties of liposomes did not change after irradiation at 25 kGy at room temperature. These results suggest that irradiation is potentially a suitable method for sterilization of MPLA-liposomes and could be applied in irradiated vaccines coupled to larger antigens.
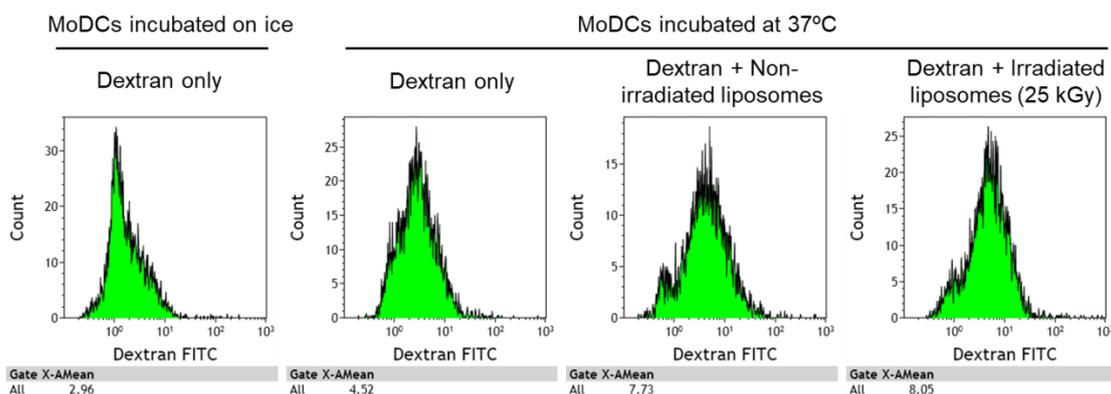


*FIG. 2: Dextran uptake by bovine MoDCs. Fluorescein isothiocyanate conjugated dextran -FITC was incubated with bovine MoDCs on ice (control) or 37°C and analysed through a flow cytometer. Antigen uptake is quantified as Gate X-Mean. Representative histograms from four independent experiments*

## *Measuring immune response to vaccines and infectious diseases*

### Development of *in-vitro* assays that measure vaccine immunogenicity using nuclear related techniques

In the framework of CRP D32033 on irradiated vaccines, the APHL has developed experimental protocols to measure the immunogenicity of vaccines during animal trials. The panel under development initially includes cytokine expression analysis by qPCR with sheep, cattle and goats as the priority. Twenty-five interleukins, and two calibrator genes have so far been optimised using cells stimulated *in vitro* for interleukin production. Cytokine production and especially that of IFN-gamma is a good indicator of cell mediated immunity induced by vaccinations against many intra-cellular and extra-cellular pathogens. To develop this assay, the use of PBMCs isolated from vaccinated animals is preferred as incubation for re-stimulation with the vaccine for several days to measure the range of cytokines produced by vaccinated animals. We harvested PBMCs from bovine and ovine blood and subsequently incubated the cells with either concanavalin A (ConA) or phorbol 12-myristate 13-acetate (PMA), both strong stimulators of immune cells. Control cultures were maintained in the same conditions as treated cells. RNA was subsequently extracted from cells and used in a qPCR assay using

SYBR green that measures the targeted interleukins. This assay has been designed to use sets of primers that amplify the targeted interleukin in both bovine and ovine samples, thus reducing cost and increasing efficiency. Activated cells displayed signature fold changes in interleukin expression as displayed with four representative targets (Fig. 3). This assay has subsequently been used for measuring interleukin expression in ruminant samples incubated with pox viruses to measure innate immune responses in virulent versus vaccine strains. The assay has also been applied to an *in vitro* lab assay that measures the immunogenicity of candidate vaccines.

**Standard practices for flow cytometry procedures at BSL-2 laboratories**

Containment of highly pathogenic and non-endemic disease agents is essential in research and diagnosis and is regulated by national legal frameworks according to international standards. Access to biosafety level (BSL)-3 facilities is not possible in many countries; furthermore, some equipment and research platforms are not always located within the high-containment facility, making it necessary to move samples out of the BSL-3 facility for further



FIG. 3: Fold change expression in ruminant PBMC stimulated using ConA or PMA when compared to non-stimulated negative PBMC

analysis. A protocol that describes how to isolate PBMCs from whole blood and CD14+ mononuclear cells from PBMCs for incubation with different infectious virus strains at a BSL-3 facility and with downstream analysis in a regular lab was therefore developed. We optimized sample procedures that should be followed when handling cells inside a BSL-3 laboratory to ensure that these cells are devoid of any infectious pathogenic agents when moved out of the facility. This protocol makes it possible to study infectious viruses in a non-endemic country or in institutions where a BSL-3 facility is not present. PBMCs were extracted from whole blood using a standard density gradient centrifugation that separates layers of blood, with monocytes and lymphocytes forming a buffy coat under a layer of plasma after centrifugation. The cells were then incubated with infectious pox viruses in the BSL-3 facility to measure innate immune markers. Two sub-protocols were further developed depending on the material required for downstream analysis. For qPCR samples, cells were harvested for RNA extraction after three days of incubation with pox virus using RLT buffer and incubated at 70°C for ten minutes before removing them from the BSL-3 facility for further analysis. This ensured that any viruses used for incubation were completely inactivated to avoid subsequent contamination. RNA extraction and qPCR was carried out in our BSL-2 facility using standardised lab protocols. Samples for flow cytometry were treated differently with a 5-day virus incubation step (protein transport blocked on day four) before surface staining and fixation at 4°C overnight (fixation of cells at 4.2% paraformaldehyde (PFA) for 18 hours) and continuing with the standard flow cytometry staining protocol after removing the samples from the BSL-3 facility. We still were able to preserve the intensity of surface staining and could carry out intra-cellular staining following fixation (Fig. 4). These
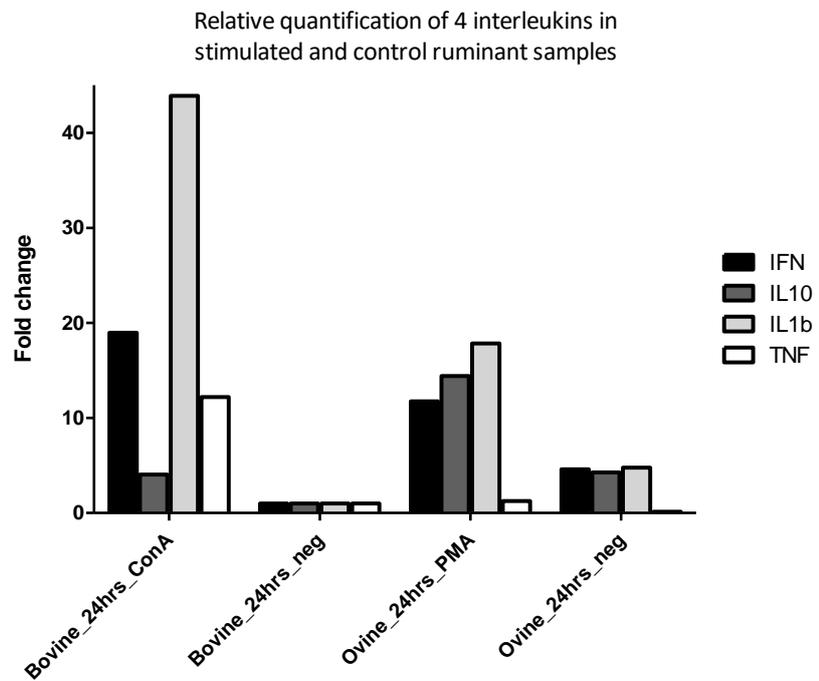
optimized protocols will aid the APHL and other laboratories to work on developing irradiated vaccines against livestock pathogens that are important to many developing nations.
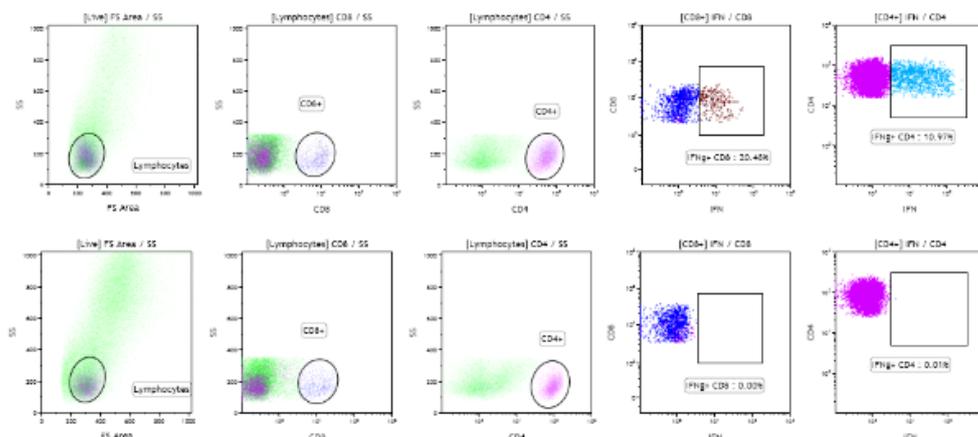


FIG. 4: Sheep leukocytes were cultured overnight in 24-well plates ($5x10^6$ cells/mL) in RPMI1640 supplemented with FBS at AGES (BSL-3). Then cells were stimulated with phorbol myristate acetate and calcium ionophore ionomycin (top panel) or without (lower panel) for four hours. Cells were harvested and stained for surface staining (CD4, CD8 and live/dead stain). Cells were then fixed overnight with 4.2% PFA (18 hours at 4°C). Next morning, cells were transported back to the APHL (BSL-2) in the same fixation solutions, where they were permeabilized and stained for cytokine IFN-gamma. Acquisition was done the same day.

## *Tackling transboundary animal and zoonotic diseases*

Transboundary animal diseases caused by various infectious pathogens (i.e. viruses, bacteria, parasites) often hamper the development of livestock economies, the trade of animals and animal products and negatively impact food security in several parts of the globe. Furthermore, some of these diseases zoonotic, causing serious illnesses and public health concerns. In many instances, these diseases are highly contagious and spread very rapidly. For this reason, the early and rapid detection of the pathogens is extremely important to implement effective control measures. The APHL has been playing an active role in the development of specific and rapid diagnostic tests and the generation of genetic data to better understand the molecular epidemiology of such infections and the evolution of the pathogens, both important to prevent the transboundary spread and their transmission to other animals and to humans.

**Peste des Petits Ruminants (PPR)**

*Rapid detection of PPR virus by targeting two virus genes*

PPR virus (PPRV), as several other RNA viruses, often undergoes genomic variations, creating a challenge in their detection by PCR, and especially by real time PCR, when new variants arise. In fact, these variants may go undetected by diagnostic tests if the mutations happen within the binding site of the PCR primers or probes. One solution to increase the chance of detecting all variants is to target multiple genes of the same pathogen. For this purpose, a one-step multiplex RT-qPCR assay for detection of PPRV by targeting two PPRV genes (the M and the NP genes) along with an internal control (beta-actin) was developed and validated (Fig. 5) by the APHL in collaboration with Member State laboratories in Cameroun, Ethiopia and Nepal. The assay was validated for sensitivity and specificity and transferred to Member State laboratories through expert missions by APHL staff and trainings organized at the FAO/IAEA Laboratories.
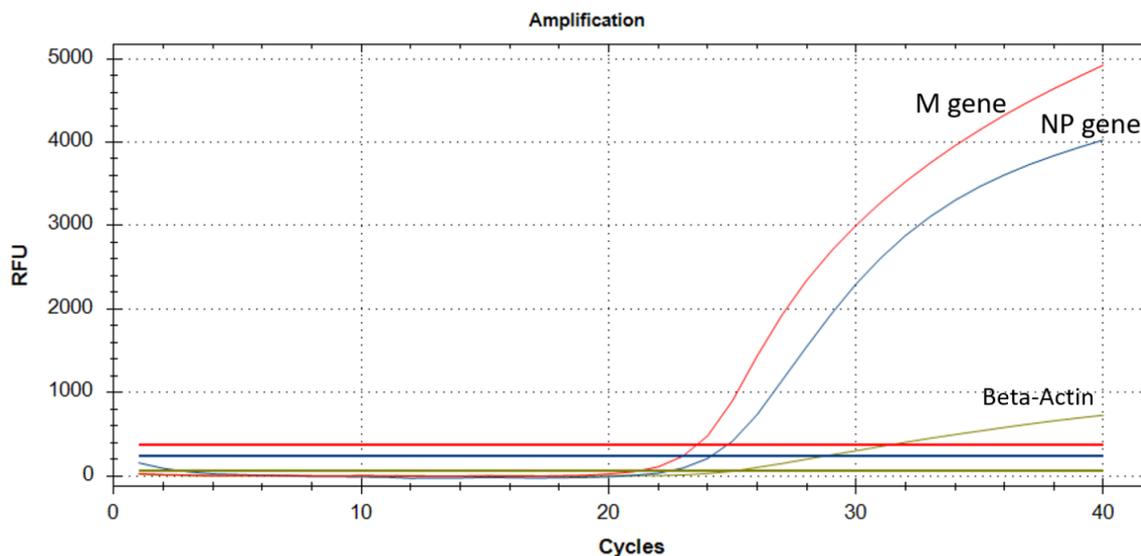
*FIG. 5: Amplification of two different genes (matrix and nucleoprotein) of PPRV RNA along with internal control gene (beta-actin) extracted from field samples*

## African swine fever

African swine fever (ASF) is a severe viral disease of swine inflicting substantial economic losses in affected countries due to high mortality rate and associated trade restrictions on pigs and pig products. ASF has a high potential for extensive and rapid geographical spread, illustrated also by recent reports in wild boar and domestic pigs in Europe and western Eurasia.

There is currently no vaccine or drug against ASF; therefore, continuous effort in the understanding of the movement and diversity of ASF virus (ASFV) isolates and research on effective vaccines are needed.

### *Molecular epidemiology of ASF in Africa*

To date, 24 genotypes of ASFV have been characterized genetically, based on the partial sequence of the P72 gene. These highlight the great diversity of ASFV isolates and the complexity for developing vaccines against ASF. Furthermore, there is no correlation between the genotypes and the pathogenicity of the virus or its serotypes. Recently, the CD2v gene has been reported to carry information enabling the determination of ASFV serotypes. The APHL has taken advantage of its repository of ASFV samples to analyse the CD2v genes of isolates from the five African countries, Cote d'Ivoire, Democratic Republic of Congo (DRC), Mali, Mozambique and Nigeria. The analysis of the partial sequences of the CD2v gene, together with a set of previously characterized ASFV serogroups, showed that eight isolates, collected in Western Africa (Cote d'Ivoire, Mali and Nigeria) and belonging to P72 genotype I, clustered with ASFV serogroup SG4, whereas four isolates from DRC, also belonging to the P72 genotype I, clustered with serogroup SG2. This concurs with previous findings that genotype I ASFV isolates belong to the serogroups SG1, SG2 or SG4. Two isolates, from DRC, did not fall within any of the known serogroups. The first one, DRC01, belongs to ASFV P72 genotype IX, and the second, DRC05, was closer to, but clearly distinct from, the M-78 isolate of Mozambique (serogroup SG3). Seven isolates from Mozambique, belonging to ASFV P72 genotype II, were clustering with the ASFV serogroup SG8, in agreement with previous findings (Fig. 6).
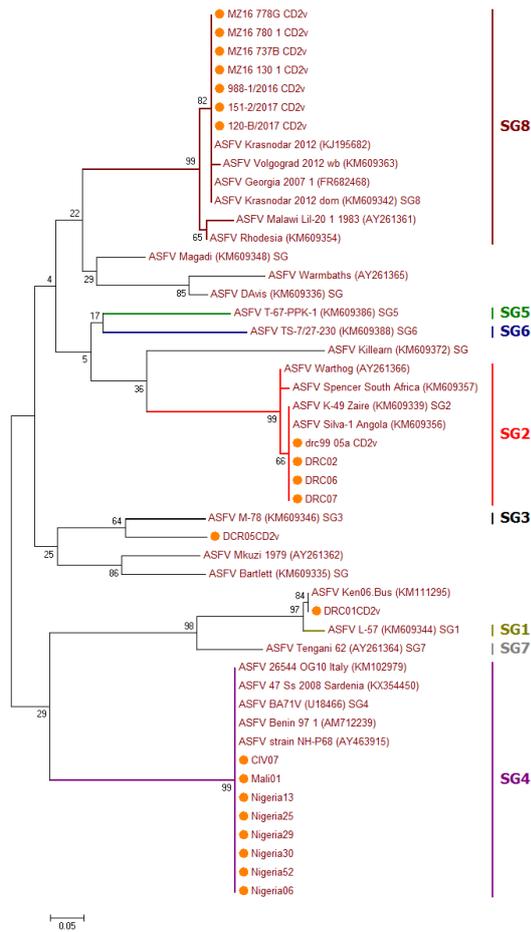
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FIG. 6: Maximum likelihood tree of ASFV CD2v protein sequences. The isolates of this study are highlighted with orange dots.

## Linking African Swine Fever Virus genetic data with outbreak information

An important gap, while retrospectively analysing pathogen genetic data, is the difficulty to associate sequences with epidemiological data to gather information on the spread of disease events or to associate the sequence variability with the severity of the disease.

The APHL has undertaken a pilot study to explore the possibility of linking ASFV genetic data with outbreak information. The P72 gene sequences of ASFV and the associated metadata were collected from the public genetic database "Genbank". The collection of the metadata was expanded using associated peer review scientific publications. To review the ASF outbreaks information, the OIE WAHIS and FAO EMPRES-I databases were used. Using this approach, only 31 sequences out of 712 isolates could be linked to disease events. This study has revealed the following major issues in creating the linkage: (1) the poor quality of the metadata available in Genbank and in publications, (2) the discrepancies between metadata in peer-review publications and those associated with the P72 sequences in Genbank, (3) the absence of records, for several outbreaks, in the OIE WAHIS and FAO EMPRES-I databases, due to low reporting in endemic countries. The minimum required data for proper linkage were: (1) the exact date (year, month, and day) and (2) the precise location (country, province, and town) of the disease event. A more stringent verification of metadata in Genbank submissions and in scientific publications, the harmonization of ASFV nomenclature (ASFV/Host/Country/Year of sample collection) and the inclusion of outbreak information in scientific publications, would facilitate such a linkage.

**Poultry infectious diseases**

### First genetic characterization of Newcastle disease viruses from Namibia

Newcastle disease (ND) is one of the most important infectious diseases of poultry globally, and is caused by virulent strains of the ND virus (NDV). The disease is endemic in Namibia but the circulating viruses have never been genotyped. In 2016, Namibia reported twelve separate outbreaks of ND in the north of the country along the Angolan border. Samples from these outbreaks were collected and the NDV was isolated at the Central Veterinarian Laboratory in Windhoek. A phylogenetic analysis using the full F gene sequence that was amplified by RT-PCR from positive samples revealed that the viruses belong to a novel sub-genotype, VIIk. This is the first genetic characterization of ND viruses from Namibia and the findings have important implications for Newcastle disease management and control in the region.

### First identification of clade E avipoxvirus in Mozambique

Fowlpox (FP) is caused by avipoxviruses, which belong to the genus Avipoxvirus in the Chorodopopoxvirinae subfamily of the family Poxviridae. The disease is of economic importance

globally because it can result in significant drops in egg production, reduced growth, blindness and increased mortality in infected flocks. FP is endemic in Mozambique but the circulating viruses have never been characterized. For this reason, samples from FP outbreaks were collected from different provinces in Mozambique by the Agrarian Research Institute of Mozambique between August 2015 and November 2016 and characterized. The outbreaks primarily affected backyard chickens and commercial laying hens although a flock of broilers and another of turkeys were also investigated. A phylogenetic analysis using an amplified fragment of the 4b protein gene sequence from FP-positive samples revealed that the majority of the samples contained virus that clustered in the well characterized subclade A1. However, two samples taken from chickens vaccinated against FP clustered in clade E, which has never before been identified in Mozambique. The presence of FP in birds vaccinated against FP viruses is worrying and requires urgent rectification of vaccination procedures and control strategies in Mozambique.

**Capripox disease**

As part of the programme to develop early diagnostic assays for transboundary animal diseases, the APHL developed an improved virus neutralization assay (VNT) for capripoxviruses. Conventional neutralization assays for the detection of neutralizing antibodies to capripoxviruses use primary cells for testing. The use of primary cells provides a challenge for their cultivation and are a source of batch variability. To address this issue, VNTs have in the past been tried using stable cell lines, such as Vero cells. However, capripoxviruses have low infectivity in Vero cells, which renders the assay inconsistent and dependent upon virus titre.

We have developed an improved VNT assay using ESH-L cells. This cell line, derived from sheep embryonic cells, showed consistent results when tested. After the initial trials, a standard operating procedure was developed and validated. This improved VNT has already helped us characterize more than 100 different positive and negative sera in our serum bank, which now provides the foundation for any serologically-based capripoxvirus assay being developed at the APHL.

## Animal genetics

### *Announcement of two whole genome radiation hybrid panels for dromedary (*Camelus dromedarius*): 5000$_{RAD}$ and 15 000$_{RAD}$*

The dromedary (one humped camel), with an estimated global population of 26.49 million, is one of the most popular domesticated animals in regions experiencing harsh climatic conditions. Dromedaries are reared mainly for milk, meat, draught and racing, and contribute significantly to the subsistence of many pastoral communities in Africa and Asia. Dromedary milk is fast gaining popularity across markets in many countries, with a good potential to improve the resilience of traditional pastoral systems. In spite of opportunities for sustainable dromedary production, systematic breeding for genetic improvement is constrained by several factors, like lack of animal identification, performance recording systems and modern genetic/genomic tools and resources. Genomic resources for camelids have been limited, except for the availability of whole genome sequences assembled to the scaffold level. However, there is a significant gap in fine-scale high resolution mapping and chromosome level assembly of camelid genomes. Availability of such tools and resources will open the possibility of whole genome scans for genetic signatures, genome-wide association studies and the development of genomic tools for breeding and improvement of camels for increased productivity and adaptability.

RH mapping has proven to be a reliable technique for producing chromosome level maps. Recent reports indicate that radiation hybrid data is extremely valuable while combining advanced sequencing and mapping procedures to produce highly accurate reference genome assemblies. In 2016, the APHL initiated the construction of dromedary RH panels and in 2017 announced the availability of 5000RAD and 15000RAD panels as permanent genetic resource for camel genome research worldwide. The overall mean retention frequency (RF) of the final set of 93 hybrids under

the 5000RAD panel was 47.7% while 90 hybrids under the 15000RAD panel had a mean RF of 39.9% (Fig. 7). The 5000RAD panel is expected to produce robust maps suitable for most purposes, while the 15000RAD panel is expected to help resolving complex questions on the Y-chromosome, intra-chromosomal rearrangements (e.g. inversions), the major histocompatibility complex region, the pseudo autosomal region and small chromosomes. This genomic resource is immediately available for use by Member States as well as by partners of the International Camel Genome Consortium, an international consortium including scientists and camel breeding associations aiming at promoting genomic studies and research on camelids with the purpose to preserve biodiversity and help farmers to improve the performance of their stock. These panels are expected to help constructing whole genome camel RH maps, assisting camelid genome assemblies and developing genomic tools for camel improvement.
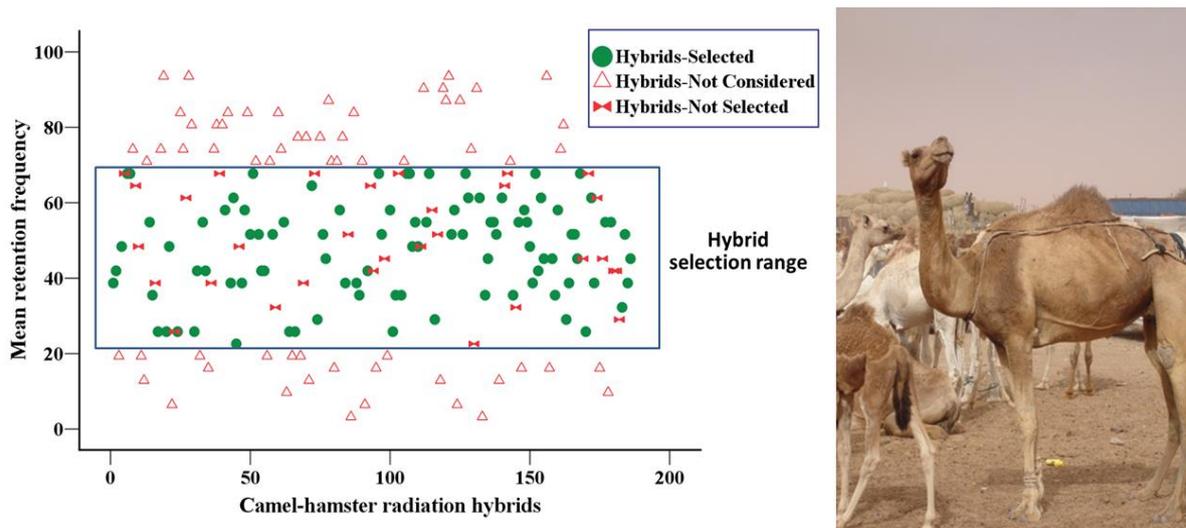


*FIG. 7: Frequency distribution of 5000RAD camel radiation hybrids based on the retention of donor (dromedary) genome*

## *Development and validation of a rapid, cost-effective genotyping tool for the detection of FecB in sheep*

The FecB mutation in bone morphogenetic protein receptor type 1B (BMP15R1B) was the first major gene widely attempted for marker assisted introgression to improve litter size in sheep. The mutation (FecB++) induces maturation of ovarian follicles by increasing the sensitivity of the follicles to follicle stimulating hormone (FSH), thus resulting in a higher ovulation rate and litter size. By transferring the mutant allele through conventional crossbreeding, it is possible to achieve a significant increase in litter size in a single generation. The effect of the FecB mutation on ovulation rate, litter size and ewe productivity essentially depends on three major factors: donor and recipient genetic background, production system type and potential maternal effect of recipient ewes. In general, FecB introgression has been found to be advantageous in smallholder sheep production systems, particularly for meat production.

DNA marker based genotyping tools help improve the efficiency of back crossing and inter-crossing in a marker assisted introgression program. The APHL has initiated the development of a rapid, cost-effective genotyping tool for the detection of FecB in sheep. A simple and accurate competitive allele method, specific PCR based genotyping, was developed and optimized. The method has now been validated and works well in at least three real time PCR platforms. Utilizing the new method, more than 1500 sheep belonging to various breeds and located across Africa, Asia, Europe and Latin America were screened. FecB was found in Indian and Indonesian sheep as expected. Interestingly, all sheep populations from Bangladesh were found to possess FecB with the frequency ranging from 33.3% to 95.5% (Fig. 8). Sheep populations from eastern and northern Bangladesh showed good genetic potential for improved prolificacy and meat production.

FIG. 8: Competitive allele specific PCR based FecB genotyping (top); frequency of FecB genotypes (bottom left) and FecB alleles (bottom right) among Bangladeshi sheep populations
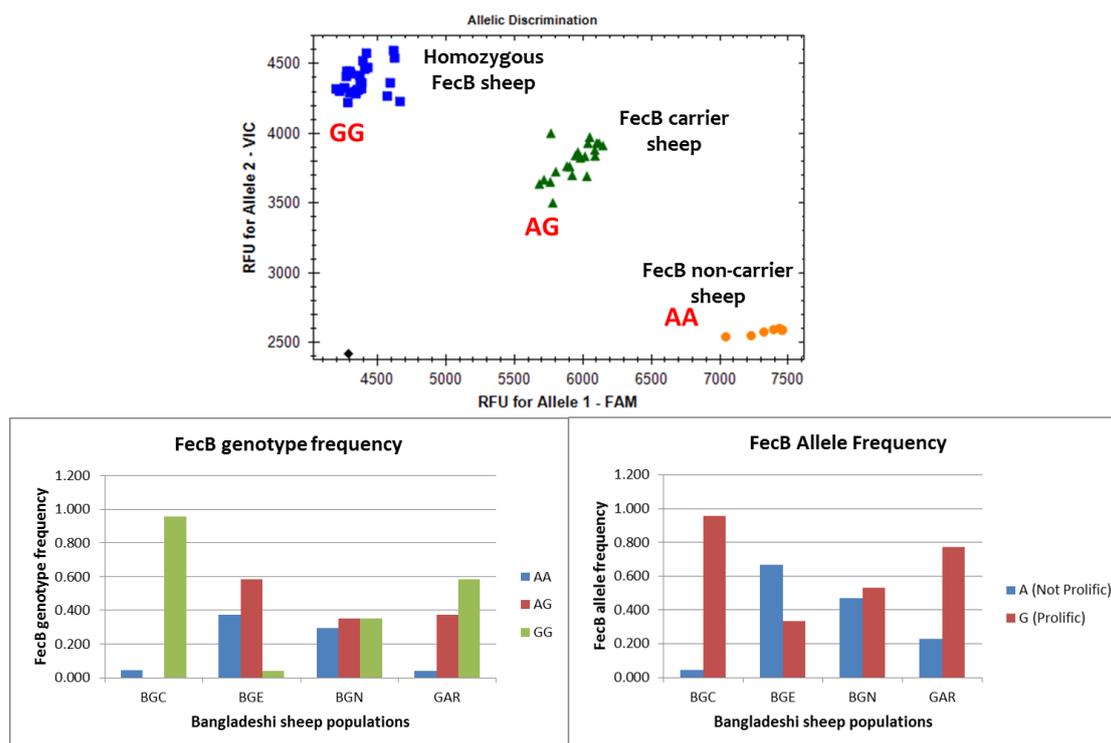
## *Implementing the Global Plan of Action for Animal Genetic Resources*

In continuation of Joint FAO/IAEA Division support towards implementing the Global Plan of Action for animal genetic resources (AnGR), the APHL supported IAEA technical cooperation projects (TCPs) on genetic characterization of native cattle, sheep and goat breeds in Sudan (SUD5036) and indigenous guinea fowl populations in Burkina Faso (BKF5017).

### Genetic characterization of indigenous Sudanese cattle, sheep and goat breeds

Sudan possesses the largest cattle population in Africa that are largely classified into two groups: Nilotic cattle and north Sudan zebu cattle. North Sudan zebu cattle include several ecotypes, like Kenana, Butana, White Nile, Baggara, Foja, Qash, Arashie cattle, Red Um Bororo, Ingessana cattle and Sudanese Fulani. Kenana and Butana are considered to have good potential for milk production as compared to other cattle populations/ecotypes. However, required information on genetic potential of these animals, especially genetic variability, level of inbreeding, physical and phenotypic characteristics, etc. is largely lacking. A total of 232 blood samples were collected from five major ecotypes of north Sudan zebu (Butana, Kenana, Foja, Baggara, Arashie) and Butana X Kenana crossbreds. All six populations were analysed by sequencing the control region (D-loop) of the mitochondrial genome and genotyping 27 microsatellite marker loci. Similarly, 141 samples from three indigenous goat breeds of Sudan (Nubian, Desert, Red Sea Hills) and 288 samples from six sheep breeds were analysed for multi locus microsatellite and mitochondrial variations. With APHL scientific and technical support for the genotyping and sequencing analysis, the Animal Resources Research Corporation (Ministry of Science and Technology) of Sudan was able to complete molecular genetic characterization of cattle, sheep and goat biodiversity for future, optimized breeding strategies.

### Genetic characterization of indigenous guineafowl populations in Burkina Faso

Guineafowl is an important backyard poultry species in Africa, providing livelihood and nutritional security in rural areas. Local guineafowl populations in Burkina Faso remain largely under-utilized due to lack of sufficient information on their genetic and production characteristics. The APHL has now

designed and developed five sets of DNA marker panels for molecular characterization of guineafowl. All five sets, involving 18 different markers, were optimized for automated genotyping. A total of 192 guineafowls located in six regions of Burkina Faso (Tenado, Dori, Gaoua, Tenkodogo, Fada and Ouagadougou) was genotyped to complete the evaluation of genetic diversity and population structure. The results of this molecular characterization will help to classify the guineafowl populations into different groups based on their genetic characteristics. These genetic groups will subsequently be tested for their fertility and growth performance under farmers' field conditions to identify the most suitable ecotypes under prevailing guineafowl production systems.

## CAPACITY BUILDING

### Genetics Laboratory Information and Data Management System (GLIDMaS)

An online training course on "Genetics Laboratory Information and Data Management System (GLIDMaS)" was held from 20–22 December 2017. A total of eight participants from four countries (Bangladesh, India, Pakistan and Sri Lanka) participated through Webex platform. The training course included demonstration of various modules of GLIDMaS, with focus on manual data entry, editing, importing multiple datasets using spreadsheet, search data and create reports. The participants were provided with a copy of the software and were individually supported on installation and hands on training. It is expected that the online training will help the participants to use GLIDMaS for managing genetic repository, laboratory resources and large volumes of molecular genetic data. Usage and application of GLIDMaS will help Member States to harmonize livestock genetic data and enhance their data management capabilities on livestock biodiversity.

### Emergency response to enhance Member State capacities in the diagnosis and control of highly pathogenic avian influenza virus (H5 subtype) in Africa and Europe

The highly pathogenic avian influenza (HPAI) virus belonging to the H5 subtype continues to circulate in wild birds and poultry in Asia, Europe and Africa causing huge economic losses to the poultry industry and, in developing countries especially, threatening food security. The virus is constantly evolving and reassorts its genes with different type A avian influenza viruses circulating in birds, giving birth to novel variants (i.e. H5N8, H5N5, H5N6) with zoonotic potential, thus raising serious public health concerns. In 2017, the H5N8 virus caused serious poultry losses in Europe and spread into sub-Saharan Africa, emerging for the first time in Uganda, Cameroon, the Democratic Republic of the Congo (DRC) and then moving further south to Zimbabwe and South Africa. Upon request of Member States, the APHL took immediate action to strengthen the preparedness of veterinary services to control the disease. This included the preparation and shipment of emergency diagnostic kits (i.e. boxes containing all the reagents and consumables necessary to address the immediate diagnostic needs of veterinary laboratories) to Uganda and DRC, assistance to national veterinary laboratories in affected or at-risk countries, such as Mozambique, South Africa and Namibia. Furthermore, a workshop was organized in Vienna with 23 participants from the European region and a training course was held in Ethiopia (hosted by the National Veterinary Institute in collaboration with the Pan African Veterinary Vaccine Centre of the African Union (AU-PANVAC, Debre Zeit) with 22 participants from Africa to apprise participants on recent advances in the detection of avian influenza as well as on the current epidemiological situation.

### Proficiency test for peste des petits ruminants virus

Like in previous years, the APHL again organized an interlaboratory proficiency test (or PT) for the molecular and serological detection of PPRV. This exercise is a confidential, blind test of the ability of a diagnostic laboratory to determine the presence of PPRV in the samples provided. The sample panel consisted of 20 samples in total, 10 for molecular (nucleic acid) testing and 10 for serological (antibody) detection. The samples for molecular testing included different PPRV strains; they were provided in several dilutions, gamma-irradiated and lyophilized prior to shipping.

Twenty-eight laboratories in 25 countries (17 in Africa, 6 in Asia and 2 in Europe) participated of this exercise. For the serology portion of the PT, 85% of participants scored 100%. For the molecular part, 95% of the participants scored 80% or higher. The APHL will assist participating laboratories in implementing corrective measures, when needed.

APHL encourages PPR diagnostic laboratories in Member States to make use of this annual exercise and test their ability to correctly detect PPRV.

### *Technical field support missions to build capacity in veterinary diagnostic laboratories*

APHL has been actively involved in the transfer of technologies to Member States and support their activities to improve animal production and health. Three field support missions were undertaken to install and calibrate critical equipment, train on rapid diagnosis of animal and zoonotic diseases, improve diagnostic skill and animal vaccines production.

**Mongolia**

In support of TCP MON5023 and of the VETLAB Network, APHL staff travelled on expert mission to Ulaanbaatar, Mongolia on 21-27 May 2017 to provide training on molecular diagnosis of PPR. The molecular multiplex method transferred to the State Central Veterinary Laboratory (SCVL) in Mongolia enabled the rapid detection of the recent PPRV outbreaks among livestock and wildlife populations in the country. The mission was in continuation of support provided to the veterinary laboratories of the country in the application and validation of molecular diagnostic and typing methods for the detection of pathogens in small ruminants and swine. The assay performance and workflow was monitored by the expert and staff were trained on data analysis. Installation and operation of ELISA readers for regional laboratories was demonstrated. Furthermore, staff of the Institute of Veterinary Medicine (IVM) was trained on whole genome sequence data analysis workflow, including NCBI blast, annotation and phylogenetic tree construction using free software.

**Myanmar**

A field mission to Myanmar was organized in December. The mission focused mainly on strengthening the veterinary laboratory capacity in the country on transboundary animal diseases, with special reference to FMD. Three laboratories were visited, the BSL2-FMD laboratory in Naypyidaw, and the FMD vaccine production and the diagnostic laboratories in Yangon. Guidelines and advice were provided on the expansion of the diagnostic service portfolio, with particular reference to the rapid diagnosis of PPR, FMD and viral swine diseases such as ASF, classical swine fever (CSF) and PRRS. The priorities and the main challenges to improving the quality and quantity of the FMD vaccine were also discussed. The APHL is currently assisting these laboratories to improve the diagnostic service for PPR and the national surveillance system for avian influenza H9N2, a virus that is causing serious problems to the poultry industry of the country.

### *Meetings*

**Technical meeting of veterinary laboratory directors in Asia and Africa participating in the VETLAB Network** and

**Second Research Coordination Meeting of CRP D32032 on Early Detection of Transboundary Animal Diseases to Facilitate Prevention and Control through a Veterinary Diagnostic Laboratory Network**

The meetings, supported by the African Renaissance Fund and the IAEA's Peaceful Uses Initiative to strengthen animal disease diagnostic capacities in Africa and Asia, took place on 8-11 August 2017 in Vienna, Austria

Veterinary laboratories directors from 18 VETLAB partner laboratories in 17 African and Asian countries (Bangladesh, Botswana, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of the Congo, Ethiopia **[**NAHDIC and NVI], Lao PDR, Mali, Mongolia, Mozambique, Namibia, Nepal, Senegal, United Republic of Tanzania and Zambia) attended the meeting (Fig. 9) to update on their

progress, achievements and challenges in 2016/2017 and to formulate work plans for 2018. The main highlights of the meeting were as follows: (1) each individual laboratory has broadened its scope in pathogen detection, including in some cases the use of advanced technology, such as multi-parametric pathogen detection; (2) VETLAB partner laboratories from DRC, Ethiopia, Namibia and Senegal are now using sequencing to improve disease diagnostics and to further understand the spread of pathogens; (3) most VETLAB partner laboratories are pushing toward implementation of a quality management system (QMS) and ISO 17025 accreditation. QMS is already in place in several VETLAB partner laboratories that have also appointed a quality officer. Those most advanced have increased their number of accredited tests under ISO 17025; (4) local visibility and credibility has improved for most laboratories, enabling some VETLAB partner laboratories, such as NAHDIC and NVI (Ethiopia) and the Laboratoire National d'Élevage in Burkina Faso), to receive funding from local authorities for laboratory refurbishment and expansion; (5) Cameroon, DRC and Mongolia notified of new outbreaks in 2016/2017, based on the results of local VETLAB partner laboratories.

The assembled directors also discussed their major constraints in disease diagnosis and management. Common strategies and solutions were identified to strengthen the VETLAB partners' laboratory capacities to establish and reinforce their roles in the national and regional control strategies against transboundary animal diseases.

The meeting was held in parallel with the second Research Coordination Meeting of the VETLAB CRP D32032 (Early Detection of Transboundary Animal Diseases to Facilitate Prevention and Control through a Veterinary Diagnostic Laboratory Network). The veterinary laboratory directors interacted constructively with the CRP experts and provided decisive input to the CRP work plan. The partner laboratories supported the proposed objectives and the new work plan of the CRP to promote the production of secondary standards for the implementation of quality systems. Moreover, agreement was made on strategies to promote the use of multi-parametric detection of pathogens and the use of sequencing.



*FIG. 9: Participants of the VETLAB technical meeting with directors of Asian and African veterinary laboratories*

## *Training courses*

### Regional Training Course on Nuclear and Nuclear Related Techniques for Early and Rapid Detection and Differentiation of the Middle East Respiratory Syndrome in Camels

24 April to 5 May 2017, FAO/IAEA Laboratories, Seibersdorf, Austria

Upon request of the veterinary authorities of Member States from the Middle East region, the APHL hosted a training course on laboratory techniques for early and rapid detection and differentiation of Middle East respiratory syndrome coronavirus (MERS-CoV) infections in camels. Middle East respiratory syndrome (MERS) is a severe infectious respiratory disease in humans caused by a coronavirus (CoV). The disease is mainly present on the Arabian Peninsula, where the majority of human cases are notified. Dromedaries have been shown to be reservoirs of the virus. Nineteen participants from seven Member States attended the meeting. The training course covered the epidemiology of the disease (review of both human and animal infections), techniques used for detection of specific antibodies against MERS-CoV in camels, and molecular techniques used for detection of viral RNA. The participants also received practical training on basic bioinformatics and epidemiological considerations for the use of the assays in the field. Lecturers included five international experts from the Faculty of Veterinary Medicine, Vienna, the Charité – Universitätsmedizin, Berlin, the University of Cairo and FAO, Rome.

### Regional Workshop on Advanced Techniques for Detection and Differentiation of Avian Influenza Viruses, in the Light of Current Outbreaks of Avian Influenza in Europe

11-22 September 2017, FAO/IAEA Laboratories, Seibersdorf, Austria.

Twenty-three participants, from 23 Member States of the European region, attended the workshop. The event was organized as a response to outbreaks of highly pathogenic avian influenza in 2016 and 2017, predominantly caused by the novel subtypes H5N8 and H5N6 affecting, Asia, Europe and Africa. Mains topics covered were: advanced diagnostics of avian influenza viruses (techniques used for screening, confirmation, direct pathotyping and use of techniques for differentiation of vaccine from field virus strains); application of diagnostic techniques in surveillance and early detection, including tracing migration of wild birds using stable isotopes; advanced bioinformatics and genetic characterization (conventional versus whole genome and next generation sequencing); and laboratory networking in the support of the surveillance and control programmes.

### Transboundary Animal Diseases: Multiple Pathogens Detection

25 September to 6 October 2017, FAO/IAEA Laboratories, Seibersdorf, Austria

Twenty participants from VETLAB partner laboratories in 19 African and Asian countries (Bangladesh, Botswana, Burkina Faso, Cameroon, Chad, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Ethiopia, Kenya, Lao P.D.R., Mali, Mongolia, Mozambique, Namibia, Nepal, Senegal, United Republic of Tanzania and Zambia) attended the training course (Fig. 10). Its purpose was to strengthen the veterinary diagnostic and research capacities of Member State laboratories in the differential diagnosis of infectious animal diseases using multi-parametric pathogen detection technologies. These techniques make it is possible, using a single reaction vessel, to detect and differentiate pathogens causing infectious diseases that have similar clinical signs. The training consisted of lectures on the principles and practical sessions on the application of multi-parametric technologies for the diagnosis of the major transboundary and zoonotic animal diseases threatening livelihoods and health in several Member States in Africa and Asia. The training was provided by experts from the Institut de recherche pour le développement (IRD), France; the Friedrich-Loeffler-Institut, Germany; the Luminex Corporation, the Netherlands, and the Joint FAO/IAEA Division.

*FIG. 10: Participants at the VETLAB training course on transboundary animal disease diagnosis in Seibersdorf*

**Early Detection and Characterization of Transboundary Animal and Zoonotic Diseases**

23 October to 3 November 2017, National Veterinary Institute, Debre Zeit, Ethiopia

To mitigate the spread of TADs within and between countries, as well as the spillover of zoonotic diseases to humans, the capacity of veterinary diagnostic laboratories must be improved through a constant update of new diagnostic tools developed to identify and track the evolution of the pathogens responsible for the diseases. The main purpose of this training was to strengthen the participants' ability to detect and conduct surveillance of major viral and bacterial pathogens affecting poultry and ruminants, including those of a zoonotic nature, as well as to perform their corresponding epidemiological studies. Twenty-two scientists from VETLAB Network laboratories in 16 African countries (Botswana, Burkina Faso, Cameroon, Chad, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Ethiopia, Kenya, Mali, Mozambique, Namibia, Senegal, United Republic of Tanzania, Zambia and Niger/PANVAC) attended this course. Training consisted of lectures and practical sessions on the application of molecular and serological diagnostics, differential diagnostics and molecular epidemiology for HPAI, ND, and infectious bronchitis in poultry, and Rift Valley fever, Brucella and pox diseases in ruminants and camels. The trainers were from the National Institute for Communicable Diseases, South Africa; the Istituto Zooprofilattico Sperimentale delle Venezie and the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise in Italy; the Pan African Veterinary Vaccine Centre of the African Union, Ethiopia; the National Veterinary Institute, Ethiopia; and the Joint FAO/IAEA Division.

**Laboratory diagnosis of transboundary animal and zoonotic diseases in Southeast Asia**

6-17 November 2017, National Centre for Veterinary Diagnosis, Hanoi, Viet Nam.

Twenty participants from veterinary laboratories in Lao PDR, Myanmar, Thailand and Viet Nam attended this training course, aimed at strengthening the diagnostic capacities of national veterinary laboratories in Southeast Asia for the identification and classification of pathogens responsible for

animal infectious diseases of major interest, including rabies, capripox, leptospirosis and animal clostridiosis. The course was organized jointly by the National Centre for Veterinary Diagnosis in Hanoi, the Joint FAO/IAEA Division and the IAEA's Department of Technical Cooperation and included seminars and practical sessions on gel-based PCR, real time PCR, 16S rRNA sequencing, and immunofluorescent and ELISA tests to rapidly detect and type disease pathogens. The trainers were from the Australian Animal Health Laboratories-Geelong; the Australian Veterinary Laboratory Service, Brisbane; the National Institute of Animal Health, Bangkok, and the Joint FAO/IAEA Division.

*Fellowship and internship training*

In 2017, the APHL hosted 11 interns/fellows in the following areas:

| Name | Country | Status | Duration | Topic |
|---|---|---|---|---|
| **Gaggl**, Anna | Austria | Intern | 2 months | Screening of radiation hybrid panel for mapping camel genome |
| **Hamed**, Hiba | Sudan | Fellow | 3 months | Genetic characterization of Sudanese indigenous cattle and goat breeds using DNA markers |
| **Kenny Uranoli**, Betty | Papua New Guinea | Fellow | 2 months | DNA marker based molecular characterization of livestock |
| **Magboul**, Mutaz | Sudan | Fellow | 3 months | Evaluation of Sudanese sheep for prolificacy using DNA markers |
| **makgwa**, Andrew L. | Botswana | Fellow | 3 months | Laboratory diagnosis of transboundary animal diseases using molecular and immunological techniques |
| **Makun**, Hussaina | Nigeria | Fellow | 3 months | Real time PCR based species detection and genetic diversity analysis of Haemonchus parasites |
| **Marambe**, Stephen T. | Bangladesh | Fellow | 1 month | Laboratory diagnosis and molecular epidemiology of transboundary animal diseases |
| **phonthasi**, Sengxay | Lao PDR | Fellow | 3 months | Laboratory diagnosis of transboundary animal diseases using molecular techniques |
| **Raschia**, María A. | Argentina | Fellow | 3 months | Bioinformatics analysis of genotypic data on host genetic resistance against gastro-intestinal parasites in sheep |
| **Traore**, Amadou | Burkina Faso | Scientific visit | 2 weeks | Bioinformatics analysis of short tandem repeat data to assess population structure in livestock |

| Name | Country | Status | Duration | Topic |
|------|---------|--------|----------|-------|
| **Traore**, Fabiola | Burkina Faso | Fellow | 3 months | Molecular genetic characterization of guineafowl populations using nuclear and extra-nuclear DNA markers |

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## VETLAB NETWORK

The APHL continued to provide important contributions to the VETLAB Network, a network consisting of national veterinary diagnostic laboratories located in 44 African and 19 Asia and Pacific Member States. The VETLAB network is coordinated by the APH Subprogram and supported through FAO/IAEA programmatic activities as well as by South Africa through the African Renaissance Fund and USA and Japan through the IAEA's Peaceful Uses Initiative.

A series of VETLAB Network events aiming at building diagnostic capacities and sharing information and expertise were contributed by the APHL. The events consisted in the Annual VETLAB Laboratory Directors Meeting (Vienna, Austria, August 2017), the training course on multiple pathogens detection (Seibersdorf, Austria, September 2017) and the training course on transboundary animal diseases: early detection and characterization (Debre Zeit, Ethiopia, October 2017). The APHL also provides continuous on-site and on-line support to partner laboratories on disease diagnosis, outbreak investigations, laboratory troubleshooting, etc. and contributes to the VETLAB Network Bulletin, a forum for participating laboratories and other stakeholders to communicate and exchange knowledge/information, to showcase achievements and to share expertise within the VETLAB Network.

## EXTRA-BUDGETARY SUPPORT

AFRICAN RENNAISANCE FUND: Improvement of veterinary laboratory capacities in South Saharan African countries. Funded by the Department of International Relation and Cooperation of the Republic of South Africa.

IAEA's PEACEFUL USES INITIATIVE: The improvement of and capacity building in nuclear and nuclear related animal disease diagnostic capacities of veterinary laboratories in Africa. Funded by the United States' Department of State and by the Government of Japan.

# THE FOOD AND ENVIRONMENTAL PROTECTION LABORATORY

## EXECUTIVE SUMMARY

The Food and Environmental Protection Laboratory (FEPL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture provides assistance to Member States in implementing food control systems to ensure the safety and quality of the food supply, safeguarding consumer health and helping to facilitate international trade. Technical support is provided for food provenance and authenticity determination and for contaminant control systems, focusing on the questions "is my food safe?", "am I eating what I think I'm eating?", and "am I getting what I paid for?". This support underpins food safety and traceability systems and combats economic loss through the illegal production and marketing of counterfeit and adulterated products. Activities include applied research and the development, validation, transfer and application of nuclear and related methods for testing foods. The application of these technologies and methods in Member States is supported by the development and provision of technical protocols, advice and guidance, training both in the FEPL and in Member States, and providing input for the development of international standards.

Research and development achievements in 2017 included the development and evaluation of analytical methods to underpin food traceability systems, for food authentication, and to control residues and contaminants in food. The focus was on important commodities in international trade and targets for fraudulent practices such as counterfeiting or adulteration. Method development encompassed both rapid screening methods and more sophisticated techniques, in order to provide Member States with the options needed for their particular food control systems. Rapid screening methods utilizing hand-held or portable instruments were developed for the detection of extra virgin olive oil adulteration, and for the classification of oranges and of edible bird's nest by geographical origin, using Fourier-transform infrared spectroscopy (FTIR). Other methods employing more sophisticated measurement techniques included a method for the detection of gelatin adulteration of edible bird's nest by metabolomics using high-resolution mass spectrometry, an improved procedure for the isotope analysis of the non-exchangeable hydrogen in mono and disaccharides that allows the detection of added $C_3$ and $C_4$ sugar products in foods and beverages susceptible to adulteration, and a method for verification of the geographical origin of Basmati rice by trace element analysis. All of these methods included advanced chemometric techniques for data analysis and interpretation. Methodology for food contaminant control included the development of a multi-residue method for the determination of selected pesticide residues in vine leaves, which was then applied to estimate withholding periods for some important organophosphate pesticides in a joint study with Syria, and to study different calibration methods used in pesticide residue analysis to provide guidance for Member States. The results of the R&D programme are made available through scientific publications, online method protocols and via laboratory networks such as the Red Analitica de Latinoamérica y el Caribe (RALACA) and those involved in technical cooperation and research projects

The FEPL continued to work as a research partner in the EU 7th Framework integrated project 'FoodIntegrity', and in the EU Horizon 2020 project 'Authent-Net'. The FEPL coordinated and provided technical input to two coordinated research projects (CRPs) on food traceability and authenticity, involving approximately thirty countries.

The results of FEPL research were presented at six international conferences, and the FEPL was represented in the scientific committees for three major international conferences on food safety. The laboratory interacted extensively with Member States to provide advice and information, for example through the provision of technical advice to the Seychelles Public Health Laboratory on mycotoxin analysis, to the Quality Control Council (QCC) of the United Arab Emirates on confirmation of authenticity of honey, and to Sri Lanka on authenticity testing and adulteration detection in tea. Input was provided to a meeting on the use of edible insects as a protein source. The FEPL also contributed

as a member of the UK's Food Authenticity Methodology Working Group, and in evaluating potential FAO reference centres on antimicrobial resistance.

The FEPL provided technical management for thirteen national and five regional TCPs in 2017. More than 250 scientists, analytical chemists, laboratory personnel and food inspectors from more than 50 countries were trained through these activities. The FEPL hosted two interns, one fellows, one scientific visitor and one visiting scientist during 2017. The FEPL continued to provide technical backstopping and advice to the RALACA network of food safety laboratories in Latin America and the Caribbean and was represented in the Global Food Safety Partnership, providing input to the food safety technical working group and the laboratory capacity working group.

Publications by FEPL staff in 2017 included a special issue of the Elsevier journal 'Food Control', comprising 29 peer-reviewed research papers on 'Food safety and quality: applications of nuclear and related techniques', seven papers in peer-reviewed scientific journals, six conference papers, abstracts or reports, and two book chapters.

## STAFF

| Name | Title |
| --- | --- |
| **Cannavan**, Andrew | Laboratory Head |
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| **Maestroni**, Britt Marianna | Food Scientist |
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| **Vaughan**, Amber | Intern |
| **Centonze,** Valentina | Intern |

## MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The research and development activities in the Food and Environmental Protection Laboratory (FEPL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture focus on the development or adaptation of analytical methods to help Member States to improve their food control systems. The issues of importance to Member State governments, regulators, industry and ultimately consumers can be summarized in the three questions, "is my food safe?", "am I eating what I think I'm eating?", and "am I getting what I paid for?". The analytical methods developed in the FEPL and transferred to Member State laboratories, therefore, include methods for the detection and quantification of chemical residues and contaminants in food (e.g. pesticides, veterinary drug residues), for testing various criteria related to the authenticity of foods, such as confirmation of stated geographical origin, production technique (e.g. organically produced foods), quality (e.g. extra virgin olive oil as opposed to processed olive oil) and adulteration (e.g. dilution of extra virgin olive oil with vegetable or nut oils, dilution of honey with corn syrup).

Various analytical techniques can be applied to support food safety, authenticity and traceability systems. Key analytical techniques include stable isotope ratio measurements to gain information from the naturally occurring isotopic fingerprint of the food, which can be related to environmental and climatic signals during food production, metabolomics (molecular fingerprinting) using high-resolution mass spectrometry to differentiate between authentic and non-authentic samples, and trace element fingerprinting. Such techniques applied singly for specific purposes or in combination can provide reliable information on the food samples tested and, when incorporated in overall food control systems, can improve confidence in the integrity of the food supply, allow corrective actions to be taken when there is a food safety incident, and help to combat food crime. Although these techniques are vital, they are often expensive to apply and require highly trained personnel. Our recent approach, therefore, is to develop applications for cheaper, field-deployable, rapid screening tests that can be routinely applied at multiple points along the food chain and will highlight where there is an apparent problem with a product that does not appear to match its supposed characteristics. In this way, the probability of an unsafe, adulterated or counterfeit product reaching the consumer is greatly reduced, and decisions can be made on whether to remove the product from the supply chain or initiate further investigations using the more sophisticated analytical techniques. Method development in the FEPL encompasses both the rapid screening methods and the more sophisticated techniques, in order to provide Member States with the options needed for their particular food control systems.

### Food traceability and authenticity

#### *Rapid detection of extra virgin olive oil adulteration by 'Adulterant Screen' (FTIR-ATR)*

In 2016, the FEPL investigated and reported a method for the detection of adulteration of extra virgin olive oil (EVOO) using a hand-held 'SCIO' infrared detector. In 2017, an additional test for detection of EVOO adulteration, which could be applied as an alternative to the SCIO and also as a cross-validation test of the previously described method, was applied to admixtures of EVOO with adulterant oils (rapeseed and sunflower oil) ranging between 0 to 100% olive oil. The test uses an algorithm called 'Adulterant Screen', available in the software of the Perkin Elmer 'Spectrum Two' Fourier transform-infrared spectrometer (FTIR), which was fitted with a micro-diamond attenuated total reflectance adaptor (ATR). Slight differences between the different oil types can be detected in the infrared spectrum due to the carbonyl groups in the triglyceride (Fig. 1). These spectral differences enable detection of adulteration of extra virgin olive oil with rapeseed/sunflower oil down to 5% using the adulterant screen method.

A series of authentic EVOO samples were measured and used to generate a library of authentic samples. Single spectra of pure adulterants (sunflower and rapeseed oil) were also recorded and stored under an adulterant library. The adulterant screen method was tested using samples with

known concentrations of adulterant (rapeseed oil at 10, 20 and 30%) and also with pure EVOO. The results are shown in Fig. 2. The result for adulterated samples were 'fail' indicating the presence of adulterant, while a 'pass' result was generated for pure olive oil samples.

When a suspicious sample is scanned, the algorithm first compares its spectrum to a PCA model generated from the



FIG. 1: The spectra of extra virgin olive oil admixtures from 10% to 90% rapeseed oil (RSO)

reference (authentic) materials. This model is then augmented with each of the adulterant spectra in turn. If including a given adulterant in the model greatly increases the fit of the sample spectrum, it is likely that the adulterant is actually present in the sample.

This method is simple to use, requires only the collection of the spectra (authentic samples and the known adulterants) and doesn't require any additional statistical analysis. Therefore, it can be easily used for routine analysis as a rapid screening technique.
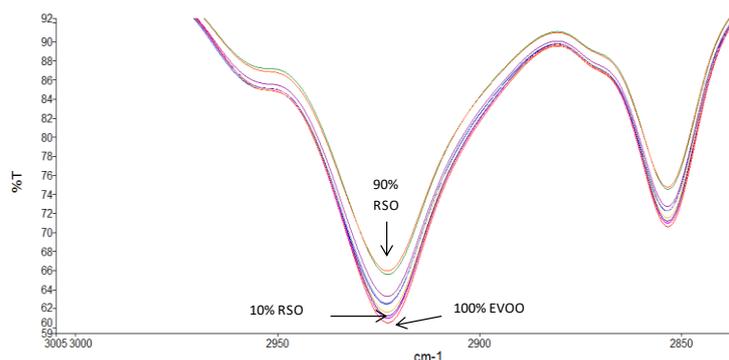
| Sample Name | Adulterant | Level | Confidence | Unidentified Com | Adulterant Screen Pass/Fail |
|---|---|---|---|---|---|
| test 70 | RSO_2 | 0.24172 | Likely | Probable | Fail |
| test 80 | RSO_3 | 0.17608 | Likely | Possible | Fail |
| test 90 | RSO_1 | 0.10448 | Likely | Possible | Fail |
| Test_EVO Pure_2 | No adulterants | - | - | Unlikely | Pass |

FIG. 2: Screenshot of 'Adulterant Screen' results for test samples (admixtures of rapeseed (RSO) and extra virgin olive oil (EVO). Test 70, 80 and 90 correspond to 30%, 20% and 10% adulteration)

### Rapid classification of oranges by geographical origin using FTIR-ATR spectroscopy and chemometrics

Oranges are one of the most important horticultural crops grown worldwide. They are of particular interest because of their high nutritional value and high contents of vitamin C and phenolics. Oranges play an important role in the human diet and the orange fruit industry is large and profitable. Their economic value makes oranges a target for misrepresentation. For example, oranges sold in Italy as high quality, high value local produce may in fact be cheaper, lower quality fruits imported from another country. This has negative implications for both industry and consumers.

We investigated the use of untargeted, quick, relatively cheap, and non-destructive spectroscopic measurements with subsequent data processing by chemometrics for classification of the geographical origin of oranges. Mid-infrared spectroscopy, FTIR-ATR, was used to analyse juice freshly squeezed from oranges grown in Italy, Spain and South Africa (150 samples).

Models were built using authentic samples from each country (80% of the samples available), and used to predict whether the rest of the samples (20%) belonged to that country. Clear separation between orange samples obtained from the different countries was achieved using linear discriminant analysis (LDA) (Fig. 3). The percentage specificity for the LDA model was 85% (Italy), 95.45% (South Africa) and 100% (Spain).

This set was further analysed using soft independent modelling by class analogy (SIMCA), to develop a one-class target classification model (Italy, class 1; Spain and South Africa, class 2) (Fig. 4). The specificity of the SIMCA model after cross-validation was greater than 93%.

This study demonstrates the great potential of FTIR-ATR spectroscopy combined with chemometrics as a rapid screening tool for the classification of oranges on the basis of their geographical origin.
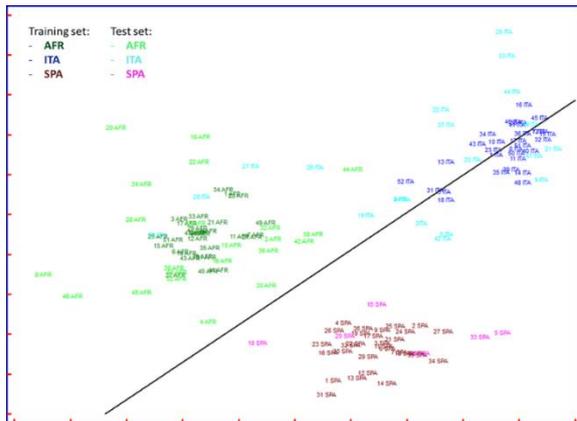


FIG. 3: LDA classification by geographical origin of orange samples from Italy, Spain and South Africa
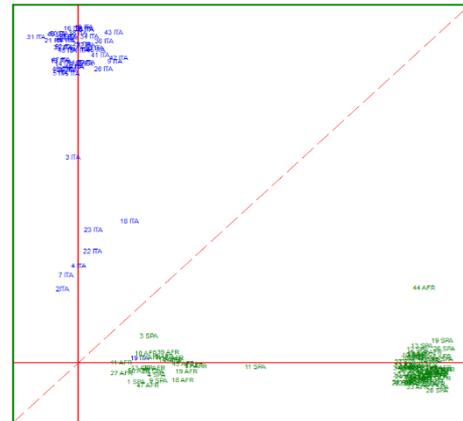


FIG. 4: SIMCA classification by geographical origin of orange samples from Italy, Spain and South Africa

## Rapid classification of edible bird's nest by geographical origin

For any food commodity, there is a greater incentive for food adulteration when the demand and prices are high, and greater opportunity when complex supply chains are involved. One food commodity that fulfils these criteria is edible bird's nest (EBN). As a rich source of amino acids, carbohydrates and mineral salts, bird's nests have been used for hundreds of years as an important health supplement in traditional medicine in south-east Asia. Examples of the use of EBN include as a treatment for malnutrition, as an immune system booster, and to enhance the body's metabolism. More recently EBN has also been used in cosmetic products.

The high demand for EBN and the limited supply has led to a lucrative market. This in turn has led to an upsurge in fake and adulterated edible bird nest products, including misrepresentation of their origin.

The aim of this research was to investigate the possibility of applying infrared spectroscopy as a rapid screening technique to differentiate between EBN from different regions. Authentic samples were obtained from seven different regions in Malaysia: Kelantan (n=15), Perak (n=11), Malacca (n=10), Negeri Sembilan (n=7), Pulau Pinang (n=7), Sarawak (n=20) and Selangor (n=32) (Fig. 5). The samples were analysed by FTIR-ATR, which permits the EBN sample to be measured without preparation of a potassium bromide disk. The measurement



FIG. 5: Sampling locations for Malaysian EBN's: Kelantan ( ), Perak ( ), Malacca ( ), Negeri Sembilan ( ), Pulau Pinang ( ), Sarawak ( ), and Selangor ( )

data were used to build models for the different regions using data-driven SIMCA (DD-SIMCA) chemometric analysis, and the models for each region were applied to predict whether the samples from all other regions could be excluded as aliens.
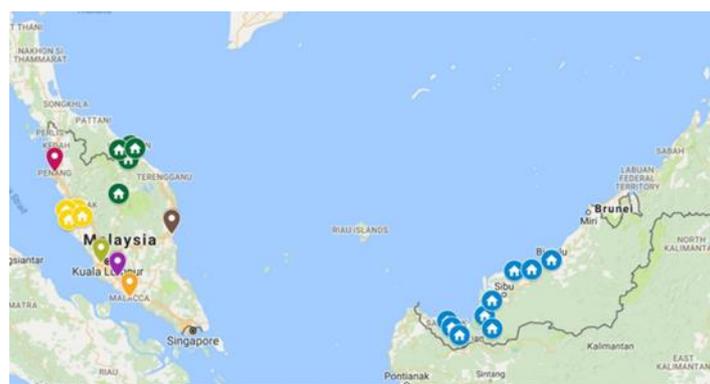
For each region, a one class target classification model was developed using authentic samples from that region. Cross-validation was performed by taking two authentic EBN samples, chosen at random from within the model region, and analysing them using DD-SIMCA to evaluate the sensitivity (the portion of correctly identified samples of the target class) of the model. Using cross-validation, the sensitivity ($\alpha = 0.05$) of the models ranged from 100% (Perak, Selangor, Sarawak) down to 67% (Negeri Sembilan) (Table 1). The specificity (the portion of objects of an alternative class that were correctly identified as members of that alternative class) of the models for each region was determined using the rest of the samples (the alternate class), which do not belong to that specific region (the target class). The specificity for DD-SIMCA models ranged from 100% (Malacca) down to 17% (Selangor) (Table 1).

*Table 1. Sensitivity/specificity obtained by DD-SIMCA for Malaysian EBN's coming from different geographical regions*

| EBN origin | Kelantan | Perak | Malacca | Negeri Sembilan | Pulau Pinang | Selangor | Sarawak |
|---|---|---|---|---|---|---|---|
| **Sensitivity (%)** | 93 | 100 | 90 | 67 | 83 | 100 | 100 |
| **Specificity (%)** | 96 | 99 | 100 | 79 | 85 | 17 | 43 |

Examples of the specificity prediction results of DD-SIMCA analysis for EBN from three of the different geographical origins are presented graphically in Fig. 6.
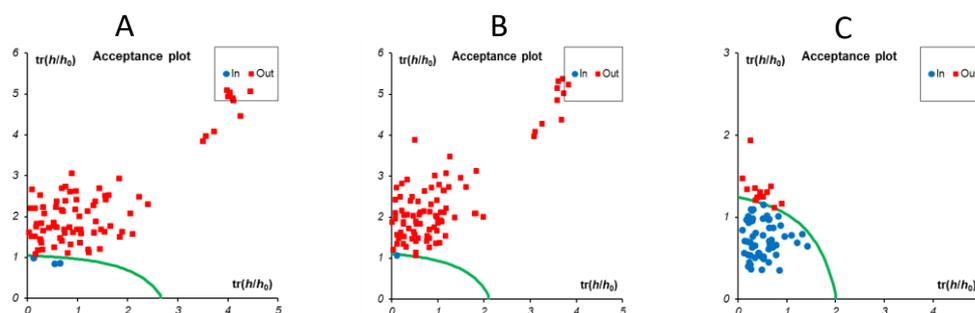


FIG. 6: Example DD-SIMCA acceptance plots for Malaysian EBN classification, showing two regions for which good specificity was obtained: (A) Kelantan vs. others; (B) Perak vs. others; and one with poor specificity: (G) Selangor vs. others (3 PCs; 95% confidence interval ($\alpha = 0.05$))

Although the number of samples used to build the models was relatively low, the sensitivity/specificity values obtained for some regions (90-100%, Kelantan, Perak and Malacca), show that there is potential for the prediction of EBN's by geographical origin using MIR-ATR spectroscopy. Further experiments with many more authentic and commercial EBN samples from different regions and countries are required to build more robust classification/prediction models and to investigate their ability to reliably classify unknown samples.

### *Detection of gelatin adulteration of edible bird's nest*

Unethical suppliers blend bird's nest with additives (gelatin, algae, agar, white jelly fungus, sugar, monosodium glutamate, isinglass, etc.) in order to boost its weight, improve its physical appearance and increase market value. There is a need, therefore, for the development of a range of analytical methods for the detection of EBN adulteration. In 2017, research in the FEPL on EBN authentication was expanded to include the detection of gelatin adulteration in EBN by untargeted profiling using ultra-high-performance liquid chromatography – quadrupole time-of-flight mass spectrometry (UPLC-QToF MS) and multivariate data analysis (MVA).

Authentic EBN samples, obtained directly from processing houses in Malaysia, and bovine, porcine and fish gelatin were analysed. Clear separation between EBN and gelatin samples was achieved using principal component analysis (PCA). In order to obtain relevant information regarding differences in the metabolic profile between EBN and gelatin samples, a set of statistically meaningful markers was selected from a loadings plot and unique marker peptides were selected for each gelatin (Fig. 7).
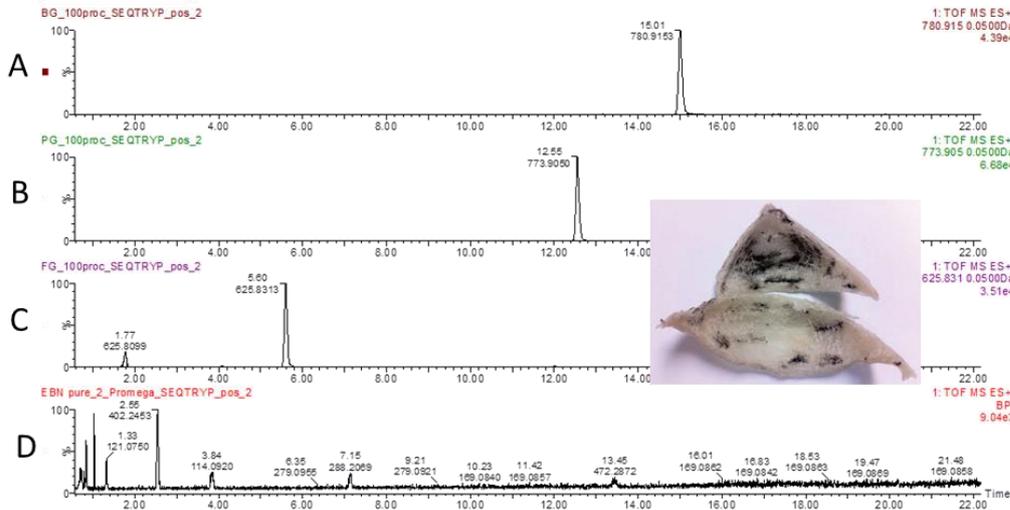


FIG. 7: Selected ion monitoring chromatograms of marker peptides in (A) bovine gelatin, m/z 780.9153; (B) porcine gelatin, m/z 773.9050; (C) fish gelatin, m/z 625.8313, and (D) EBN, containing none of the unique gelatin markers

Further research in the future will use these peptide markers of the gelatins from different species as the basis for optimisation of a simpler targeted method, which could be applied to distinguish adulterated from authentic EBN samples, as well as to identify gelatins in mixtures even at low concentration levels. This methodology shows promise as a tool that regulatory bodies could harness to combat EBN fraud.

## Isotope analysis of non-exchangeable hydrogen in mono and disaccharide sugars

An improved procedure for the isotope analysis of the non-exchangeable hydrogen in mono and disaccharides was developed in the FEPL to demonstrate the feasibility of detecting added $C_3$ and $C_4$ sugar products in foods and beverages prone to adulteration.

The detection of low value sugar syrups derived from sugar cane and corn starch is routinely achieved using stable carbon isotope analysis. Differences in the photosynthetic pathway utilised by corn and cane plants (C4) to fix carbon dioxide and that of the majority of other plant species (C3) permit the presence of the adulterant sugar syrups to be detected in fruit juices, maple syrup and honey. However, the major disadvantage of carbon stable isotope analysis is that it cannot be used to detect the addition of a $C_3$ beet sugar syrup to a $C_3$ food product such as orange juice, especially if the sugar profile is carefully matched and other ingredients, such as citric acid, malic acid and vegetable water, are added to mask the dilution.

Isotope ratio mass spectrometry can be used to detect the addition of commercial sweeteners such as beet medium invert syrup by determining the abundance of deuterium in the sugars. The natural abundance of deuterium varies widely in the biosphere due to its low mass and the large difference between the mass of the two stable isotopes 2H and 1H of hydrogen. Generally, the deuterium content of plant material decreases with increasing latitude. Since beet sugar is normally grown further from the equator than tropical fruits, beet sugar contains less deuterium than the sugars present in many fruit crops. In addition, differences in the relative rates of evapotranspiration between ground plants such as beet sugar, which has a relatively small surface area of leaves, and aerial plants such as fruit trees, result in a significant difference in the 2H/1H ratio of these species.

These differences can be exploited to detect, for example, the addition of beet medium invert syrup to fruit juices. Current methods are by conversion of the sugars to a nitro-derivative, which poses certain technical problems - the methods tend to be time-consuming, potentially hazardous, and do not readily lend themselves to high sample throughput on a routine basis, or by site-specific natural isotope fractionation nuclear magnetic resonance (SNIF-NMR), Although SNIF-NMR has been adopted by the European Union as the official method for detecting illegal addition of beet sugar to wine, the method is time-consuming and complicated.

The procedure developed in the FEPL utilizes a simple one-step reaction, with the derivatising agent N-methyl-bis-trifluoroacetamide, to substitute the exchangeable hydroxyl-hydrogen with trifluoroacetate derivatives that are sufficiently volatile to be separated and measured by gas chromatography coupled to isotope ratio mass spectrometry (GC-Cr-IRMS). The conversion of the derivatised sugars into the measuring gas is achieved using a high temperature chromium reactor that retains carbon, oxygen and fluorine whilst releasing hydrogen gas for stable isotope measurement. Deuterium chromatograms of glucose, fructose and sucrose with a quality control material (USGS70) are shown in Fig. 8.
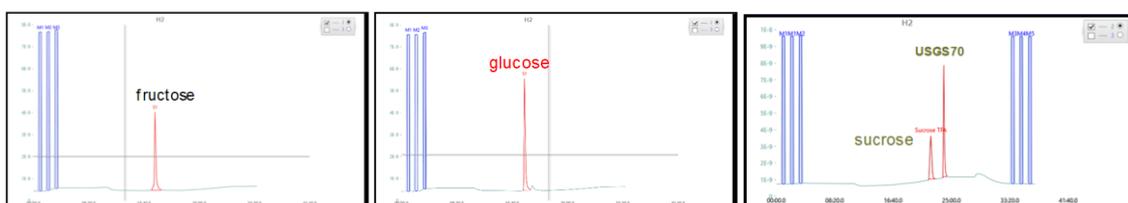


*FIG. 8: GC-Cr-IRMS deuterium chromatograms for fructose, glucose and sucrose*

The new procedure has advantages over methods using nitro-sugar derivatives and degradation products, such as hexamethylenetetramine and calcium formate, in terms of ease of use, analysis time and sensitivity. The differences between the $\delta^2H$ values of the non-exchangeable hydrogen in sugars from fruit juices and honey and those from beet and cane sugars/syrups permit the presence of these potential adulterants to be rapidly detected.

### *Verifying the geographical origin of Pakistani Basmati rice*

Mislabelling and adulteration is a problem in many areas of the food industry. It threatens the livelihood of honest traders and the rights of the consumer. For most food products the authentic item is distinguished by the absence of adulterants, by botanical/cultivar origin or geographical origin. In the case of Basmati rice the determination of authenticity is a more complex issue as it depends on both geographical origin and cultivar. Basmati is the name used for a class of rice comprising a few defined varieties grown in the Haryana, Punjab and Uttar Pradesh regions of India and Pakistan. The highly regarded properties of Basmati, such as its fragrance and flavour, cannot be emulated by growing the grain in other regions due to the unique climatic and environmental conditions around the Himalayas.

Genetic tests to establish the correct varieties of Basmati rice are well established using polymerase chain reaction analysis, but no established analytical techniques have been accepted for the characterization of the geographical origin of Basmati rice. With the increase in the consumer market for premium rice comes the risk that unscrupulous producers or distributors will attempt to increase profits by mislabelling inferior rice grown outside these regions. The FEPL collaborated with the University of East Anglia (UK) to address this issue by identifying the key variables that could be used to determine the geographical origin of Basmati rice through trace element analysis with chemometrics.

For trace element analysis by inductively coupled plasma mass spectrometry, milled Basmati rice samples were subjected to high pressure microwave digestion, diluted and an internal standard (Rh,

10 ppb) was added to each sample. The samples were analysed on a quadrupole based ICP mass spectrometer. Seventy-one elements were measured in total. After data pre-processing, 29 elements were removed leaving 42 continuous variable elemental concentrations:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 23Na | 25Mg | 26Mg | 27Al | 29Si | 31P | 33S | 39K | 43Ca | 44Ca | 45Sc | 47Ti |
| 51V | 52Cr | 54Fe | 55Mn | 56Fe | 59Co | 60Ni | 65Cu | 66Zn | 69Ga | 72Ge | |
| 75As | 76Se | 79Br | 81Br | 82Se | 85Rb | 88Sr | 89Y | 90Zr | 93Nb | 95Mo | |
| 111Cd | 127I | 137Ba | 139La | 140Ce | 146Nd | 197Au | 206Pb | | | | |

The data were then subjected to chemometric analysis using DD-SIMCA as the authentication technique. Where an alternative class is available, in this case the Basmati rice cultivated outside the accepted regions, DD-SIMCA provides the possibility to calculate the type II β error and construct the corresponding extended acceptance area, which guarantees that the risk of accepting a sample from the alternative class is not greater than β.

In this case we considered 21 authentic Basmati samples as the *target class*. The pre-processed trace element data from seventeen of these samples were used as the 'training samples'.

The acceptance area can also be tested for the reliability of identifying the class membership for the alternate class of Basmati cultivars grown outside the accepted geographical regions of Basmati rice in Pakistan. When DD-SIMCA was applied with four principal components, which should give a realistic result and avoid 'overfitting' the models, the following results were obtained (Fig. 9).
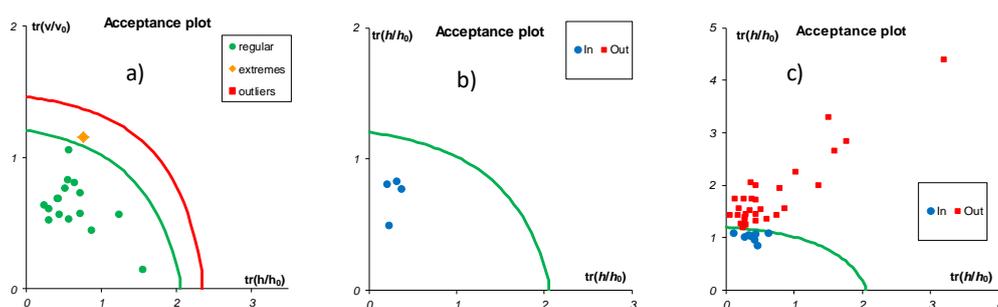


FIG. 9: The application of DD-SIMCA with four principal components to the authentication of Basmati rice cultivated in the recognized Pakistani Basmati regions. The green line is the threshold for α = 0.05 and the red line is the threshold for α = 0.01

Acceptance areas for the given α values are shown in Fig. 9a. All objects located inside the area are considered to be members of the target class. The green line corresponds to α = 0.05, which means that 1 sample out of 20 may be classified as 'extreme'. Four authentic Basmati rice samples, not used to build the classification model, were analysed and are shown plotted in the acceptance area in Fig. 9b. All four of the test samples fall within the authentic Basmati acceptance area. Based on this test set we can say that the sensitivity is 100%, i.e. there are no type 1 errors.

The results of the DD-SIMCA analysis of the alternate set of Basmati cultivars grown outside the accepted geographical regions of Basmati rice in Pakistan are shown in Fig. 9c. Seven of the 43 samples fall inside the acceptance area and are incorrectly classified as Basmati region of origin. The specificity in this case is 81%, still a useful test for Basmati origin.

## Control of residues and contaminants in food

### *Determination of selected pesticide residues in vine leaves by GC-MS/MS*

Grape (*Vitis vinifera*) is one of the most widely cultivated fruit crops in the world. It is not only the fruits that are important; vine leaves have also been used as a nutritious food in Greece and the Middle East for centuries and their popularity as a healthy food is increasing globally. To protect the crop from various pests and diseases farmers apply a range of regulated pesticide formulations that can

sometimes leave residues on the crop – these residues need to be strictly controlled. As a training programme for a TC fellow from Syria and with parallel interest from the "Red Analitica de Latino America y el Caribe" (RALACA) network, the FEPL contributed to the development and validation of a multi-residue method for selected pesticides frequently employed in grape production, in vine leaves.

A modified QuEChERS sample preparation technique previously optimised in FEPL, based on ethyl acetate extraction followed by dispersive solid-phase extraction (d-SPE) clean-up using primary–secondary amine (PSA), was selected. Sample extraction using ethyl acetate as a solvent allowed direct injection into chromatographic systems equipped with conventional detectors as well as mass spectrometers without the need for solvent exchange, making the method applicable in the Syrian laboratory and in many others throughout the world. The method had to be validated for a range of pesticides, since the applications can vary from location to location depending on the availability of the registered pesticide formulations. The method was validated, therefore, based on Codex MRLs, for 59 pesticides at 10 µg/kg, 50 µg/kg and 100 µg/kg in terms of its scope, specificity, accuracy, sensitivity, repeatability, within laboratory reproducibility and matrix effects. Key performance parameters investigated were linearity, recovery, precision, limits of detection and quantitation, and matrix effects. Recoveries for the 59 pesticides tested ranged from 60 to 110%, with relative standard deviations lower than 20%. Thirteen of the compounds, including omethoate, zoxamide and azinphos methyl, had significant matrix effects. The validated method was applied to the analysis of 27 real samples of vine leaves from Syria, which were found to be contaminated with dimethoate, diazinon, chlorpyrifos and chlorpyrifos methyl.

The method is simple, cheap and straightforward and proved to be suitable for the routine determination of pesticide residues in vine leaves. As a result of this work a poster was prepared and presented at the Sixth Latin American Pesticide Residue Workshop, entitled "Development and Validation of a Method for the Determination of Selected Pesticide Residues in Vine Leaves by GC-MS/MS".

### *Assessment of the withholding period for organophosphate pesticides applied to vine leaves*

In countries such as Syria, where vine leaves are widely consumed, it is important to assess human dietary exposure to residues of pesticides applied to vine leaves. Pesticides are applied against a wide range of insect pests, but their use must be strictly regulated. In assessing the impact of dietary exposure to pesticides various parameters must be considered and carefully evaluated, such as maximum residue limits, withholding time and dissipation rates, amongst others. These parameters are obtained either experimentally or through modelling and they vary according to the type of pesticide, type of crop and prevailing environmental conditions.

A study was initiated in collaboration with Syria on the behaviour of specific organophosphate pesticides (OP) in the field under the current agricultural practices for grape vines. The objective was to provide information for the establishment of withholding periods (WHP) for three OPs used in vineyards in Syria. The WHP is the minimum period of time that must be allowed after pesticide application before the treated area or crop can be grazed, cut for fodder or harvested. Withholding periods vary for different pesticide/crop combinations. They help to ensure that residues in the treated crop will not exceed the maximum residue limit when the crop is consumed.

Three different formulations of OPs were purchased from a local supplier and applied, at the concentrations indicated on the labels, to two different vineyards near Damascus in Syria. Entire vine leaves were randomly collected at specific time intervals after the pesticide applications (immediately after application, then at 1, 7, 14 and 21 days after treatment), brought to the laboratory and stored frozen until analysis. The method for the determination of pesticides in vine leaves described above was applied. Upon arrival at the laboratory each sample was divided into three parts. One part was stored for analysis and the other two parts were washed by immersion in either boiling water or tap water, air dried in the dark and stored pending analysis. The washing of the vine leaves is an important

domestic action when preparing the leaves as food. Residues of chlorpyrifos, diazinon and dimethoate were measured in the samples.

Results obtained from the analysis of vine leaves were plotted and statistically analysed. Chlorpyrifos and dimethoate residues followed a first order decay curve, with the slope of the regression line signifying rate of decay. The intersection of the regression line and the MRL indicated the time after application when the residual pesticide had reached the Codex MRL. For chlorpyriphos, washing the leaves had no apparent effect on the WHP whereas dimethoate showed that washing, especially with hot water, decreased the required WHP (Fig. 10). The lack of effect of washing for chlorpyrifos was attributed to its high octanol/water partition coefficient, Kow (50 - 100) compared to dimethoate that has a Kow of 5 and is much more soluble in water.
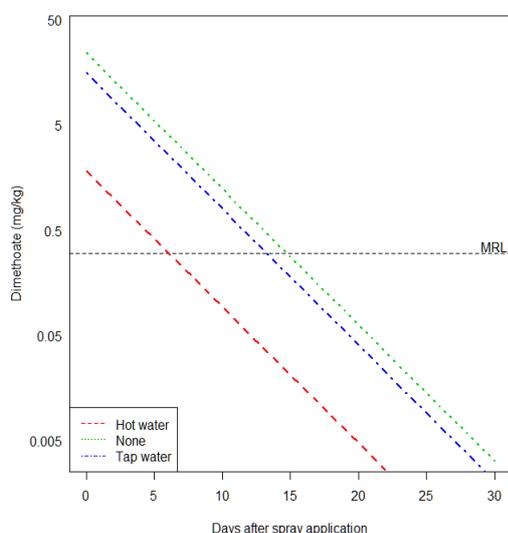


FIG. 10: Estimation of withholding time for dimethoate, curves with and without washing

The loss of diazinon did not follow a first order decay curve and analysis of those data is ongoing.

Analyses of the data will produce recommendations for the WHP between spray application and harvesting. The range of pesticides used will be useful in estimating WHPs for other pesticides. The data from this study indicate that washing with hot water removed approximately 90% of the dimethoate, which offers a practical method of decreasing the intake of that pesticide.

### *Analytical calibration: a key step for reliable data*

Analytical calibration describes a set of operations that aim to predict the unknown concentration of a target analyte through the establishment of a calibration curve. This is achieved by measuring reference standards, called the calibrators, and plotting the signals produced by the measuring instrument, usually as the area under a chromatographic peak, as a function of their concentration to establish a relationship in the form of a curve fitted by linear regression - preferably weighted regression to take into consideration inconsistency in the errors. The concentration of the analyte in an unknown can then be predicted using the established calibration curve. There are different ways of actually performing the calibration. The FEPL was requested by collaborating laboratories to investigate the use of a method known as procedural calibration as compared to other types of approaches. Chemists generally have several options for calibration: solvent calibration, matrix-matched calibration, standard additions, procedural standards calibration and the use of isotopically labelled internal standards. The choice of the type of calibration to adopt depends on several factors, but primarily on the target matrix-analyte combination. In the analysis of fresh fruits and vegetables, extracts to be analysed by chromatography coupled to mass spectrometry are often prone to matrix effects in the system, which cause signal enhancement or suppression. The calibration option selected should be as close as possible to the situation in the real samples to be analysed and the calibration standards should preferably be in an environment that is similar to the sample, to reduce the effect from the sample matrix.

In FEPL different calibration options were compared for the detection of more than 50 pesticides in vine leaves by gas chromatography coupled to mass spectrometry. The calibration curves were constructed using linear weighted regression. In almost all cases the slope of the solvent calibration curve was the highest, while the procedural calibration curve was the lowest, indicating that the signal enhancement typical of gas chromatography is well compensated by the matrix matched curve approach. On the other hand, procedural calibration helps to compensate for matrix effects and recovery losses, and also for extraction efficiency, if that is known to be an issue previously identified

during method validation studies for the particular matrix-analyte combination. The example in Fig. 11 is for zoxamide, which presents a strong matrix effect in vine leaves (131%). Compensation can be achieved with the use of matrix matched calibration or procedural calibration. However, the disadvantage for a routine laboratory is that these calibration methods considerably increase the number of samples and the analysis time, and therefore the cost. In addition, test results obtained by procedural calibration should not be corrected for recovery.
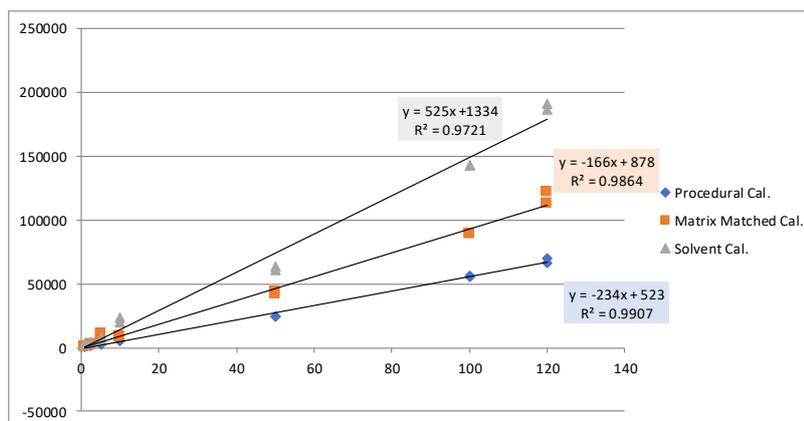


FIG. 11: Calibration curves for zoxamide

The results of this study will help analysts in Member State laboratories to select the most appropriate calibration strategy to obtain reliable results for each analyte-matrix combination. It is recommended that individual requirements are assessed case by case and the calibration approaches modified accordingly and in line with current guidelines.

## Coordinated research

In 2017, the FEPL coordinated and provided technical input to two coordinated research projects (CRPs) in the fields of food authenticity and traceability.

### *Accessible Technologies for the Verification of Origin of Dairy Products as an Example Control System to Enhance Global Trade and Food Safety*

This CRP has 14 participating laboratories in 14 countries. The 3rd research coordination meeting was held at the Vienna International Centre, Austria, 9-13 October 2017. The meeting concluded that good progress had been made by all participants in continuing collection of authentic milk samples and continuing programmes of isotopic and trace element measurement. To ensure that a primary objective of the CRP, to populate a database with high-quality data from authentic-origin, is met it was agreed that the core methods developed and used by the contract holders will be presented as standard operating procedures (SOPs) with appropriate validation data. The database structure from a successful Framework 7 European Union project on food authenticity and traceability, "TRACE", will be adopted and used as the starting point for the dairy origin database. The first joint publication from the consortium was also planned, on the validation of multi-element measurements on a common IAEA reference material milk powder (IAEA 153). The results are being collated by the Florida International University with the aim to submit the publication in the first quarter of 2018.

### *Field deployable analytical methods to assess the authenticity, safety and quality of food*

The FEPL and the IAEA's Nuclear Science and Instrumentation Laboratory (NSIL) held the inaugural research coordination meeting for a new joint international coordinated research project, 'Field deployable analytical methods to assess the authenticity, safety and quality of food', at the IAEA Headquarters from 15-19 May 2017. The project will exploit and adapt portable atomic and molecular spectroscopic screening technologies for front-line food fraud detection The meeting participants comprised eight contract holders (from China, India, Malaysia, Morocco, Russian Federation, Singapore, Sri Lanka and Uganda), five agreement holders (from Austria, Belgium, Sweden, United Kingdom and the United States of America) and five observers representing the Food and Agriculture Organisation of the United Nations (Rome), the European Joint Research Centre (Geel, Belgium),

Perkin Elmer (USA), Queen's University Belfast (Northern Ireland) and the Semenov Institute of Chemical Physics (Moscow, Russian Federation).

This joint CRP strives to close the gap between instrumental capabilities found in research labs and technologies that can be easily used by various national gate-keepers in developing countries, such as national customs authorities and food regulators. The focus of the first phase of the project is to ensure sufficient sampling, consistency of methods and data quality between participants so that the ultimate goal of generating a sustainable database in vegetable oil and milk powder atomic and molecular spectroscopic parameters can be achieved.

## EU projects

The FEPL is a research partner in two EU-funded projects; the multi-national Integrated Project, 'FoodIntegrity - Assuring quality and authenticity in the food chain', funded under the EU 7th Framework mechanism, and the Horizon 2020 project 'Authent-Net'.

### *FoodIntegrity*

The aim of the FoodIntegrity project is to build capabilities to fight food fraud and to assure the authenticity, safety and quality of European food. It involves producers, industry, retailers, public administration, control bodies, NGO's, analytical laboratories and researchers. The FEPL is active in work packages 1 (Food Integrity Network), for which Mr Simon Kelly (FEPL) has taken over leadership, 2 (Knowledge Base), 10 (Industrial Integration) and 11 (Dissemination and Knowledge Transfer). See also https://secure.fera.defra.gov.uk/foodintegrity/index.cfm.

### *Authent-Net*

Authent-Net is a two-year project that has been designed to facilitate sustainable cooperation between national and international research funding bodies in the area of food authenticity, and to improve the competitiveness of the food supply chain and the consumer confidence in it by means of better-coordinated, cost-effective R&D. The Authent-Net consortium consists of 19 partners from 12 countries and has considerable expertise in various aspects of food authenticity. The FEPL is a partner in the project, providing a more international dimension to the European partners. See also http://www.authent-net.eu/.

## Dissemination of Research Results

The results of the research and the methods developed or adapted and validated in the FEPL are made available to Member States through various mechanisms, including training courses, workshops, publications in the scientific literature and via the internet, public outreach events and conferences and symposia. The 'Food Contaminant and Residue Information System' (FCRIS, http://nucleus.iaea.org/fcris/) provides a wealth of useful data on food contaminants and residues and includes analytical method databases, which are continually updated with methods developed in the FEPL as well as others submitted by laboratories in Member States. The methods databases for veterinary drug residues and for pesticide residues were developed in response to requests from the Codex Committees on Residues of Veterinary Drugs in Food and on Pesticide Residues.

### *Conferences*

**The Jack Pearce Memorial Lecture, Belfast, UK, 24 February 2017.** Jack Pearce (1942 – 2016) was Professor of food science at Queen's University Belfast (QUB), UK, a past President of the Institute of Food Science and Technology (IFST) and a leading member of the team handling radiological food safety in the UK in the aftermath of Chernobyl. Prof Pearce was widely recognised as an outstanding scientist and strategic thinker. He collaborated with IAEA in a number of fields, notably in the follow-up to Chernobyl, and in food irradiation. Mr Andrew Cannavan (FEPL) was invited by the Royal Society of Chemistry (RSC) and the IFST to give a memorial lecture in QUB to celebrate Prof Pearce's life and work as part of the Northern Ireland Science Festival. The lecture, entitled 'Food Integrity – the

Nuclear Option' interwove aspects of the work of Prof Pearce and his colleagues with modern developments related to the application of nuclear techniques to food authenticity and safety.

**International Life Sciences Institute (ILSI) annual symposium, Brussels, Belgium, 30-31 March 2017.** Ms Britt Maestroni (FEPL) presented a keynote speech entitled 'Food Safety as a Vital Component of Food Security' at the annual ILSI symposium, which was attended by over one hundred international participants. The lecture highlighted the central role played by analytical laboratories in providing end-product testing and advice in the context of food control systems and ensuring food security. ILSI is an organization that fosters collaboration among scientists from across industry, academia and intergovernmental bodies in order to produce the best research in food safety and nutrition sciences.

**Fourth Food Integrity Conference**, **Parma, Italy 10-11 May 2017.** The FoodIntegrity Conference is the annual meeting of the EU-funded project 'FoodIntegrity - Assuring quality and authenticity in the food chain'. The theme for the 2017 conference was 'Turning science into solutions and everyday practices'. The conference had approximately 350 participants from all over the world. Mr Simon Kelly (FEPL) participated in the organisation and implementation of an interactive exercise within the 'Good risk management requirements' workshop, focusing on the economic importance, promotion and protection of protected designation of origin (PDO), protected geographical indication (PGI) and organic foods.

**Mid-term Authent-Net Conference, Parma, Italy, 11-12 May 2018**. The mid-term Authent-Net conference was held in Parma on 11-12 May 2017, with approximately 30 participants representing 19 project partners. Eleven national status reports have been developed that detail commodity and country profiles in respect to food authenticity, integrity and traceability. The kick-off meeting for the standardization process "Authenticity in the feed and the food chain - General principles and basic requirements" was held to start the process of creating consensus-based recommendations for definitions of key terms and concepts related to food authenticity, and to provide recommendations for "best practice" underlying future communication and work related to food authenticity, which will be compiled in a Low-level European voluntary standard (CWA).

**Sixth Latin American Pesticide Residue Workshop (LAPRW), San José, Costa Rica, 14-17 May 2017.** The congress was attended by almost 500 people from 44 countries. Various topics were discussed in the field of monitoring, surveillance and environment, such as monitoring of pesticide residues in fresh fruits and vegetables, organic farming and milk-derived products, passive samplers for air and water, monitoring of herbicides, and water quality in cane sugar plantations and rivers. The FEPL presented two posters on research performed with partners in several countries, focusing on a method for the determination of selected pesticide residues in vine leaves by GC-MS/MS and one for the determination of DDT soil sorption coefficients using $^{14}$C-DDT.

**Eighth International Symposium on Recent Advances in Food Analysis (RAFA), Prague, Czech Republic, 7-10 November 2017.** The eighth biennial RAFA symposium had more than 750 attendees from a broad range of food related disciplines and covered topics including authenticity and food fraud; 'omics' including foodomics; pesticide and veterinary drug residues; food forensics; mycotoxins, marine and plant toxins; bioactive compounds; nanoparticles in food; novel foods and supplements; and organic crops and foodstuffs. Mr Simon Kelly (FEPL) made an oral presentation entitled "Improving accessibility to food authentication and traceability methods in developing countries: The activities of the Joint FAO/IAEA Division's Food and Environmental Protection Laboratory". Mr Kelly also gave a presentation on "Stable Isotope Analysis to detect Food Adulteration and Fraud" in a vendor seminar by ThermoFisher.

*Special issue of "Food Control"*

A special issue of the Elsevier journal, "Food Control", focusing on selected manuscripts prepared from presentations at the FAO/IAEA Symposium on "Food Safety and Quality: Applications of Nuclear and Related Techniques", and from follow-up work discussed at the symposium and completed over the subsequent year, was published in February 2017 (sciencedirect.com/journal/food-control/vol/72/part/PB). Twenty-nine manuscripts on various aspects of food safety and control were accepted through the journal's stringent peer review process.

# CAPACITY BUILDING

The FEPL provided technical management for thirteen national and five regional TCPs in 2017. Analytical methods and technology packages were transferred and applied through the projects, training workshops held in Member States and fellowships or scientific visits in Seibersdorf. More than 250 scientists, analytical chemists, laboratory personnel and food inspectors from more than 50 countries were trained through these activities. Each of the workshops was designed with an individual focus, but all were within the framework of food safety and quality and included the protection of the integrity of the food supply chain as a holistic process, involving multiple stakeholders and requiring the application and integration of different analytical methods and technologies. Staff from the FEPL contributed directly to capacity building through a number of expert missions to Member States, including China, Costa Rica, Sri Lanka, United Arab Emirates and Uruguay. The training activities and meetings provided a platform for interdisciplinary networking between stakeholders in the "farm-to-fork" food chain and fostered the formation of a global network.

*Training course on 'Analytical methods for residues of selected pesticides'*

The FEPL, in collaboration with the Faculty of Chemistry, Universidad de la República (UDELAR), Uruguay, held this regional training course in Montevideo, from 13-17 February, and in Paysandú from 18-24 February 2017. Eighteen participants from ten countries - Argentina, Brazil, Chile, Costa Rica, Cuba, Ecuador, Guatemala, Panama, Paraguay and Uruguay - participated in the course.

The main purpose of this training was to develop the skills of the participants in the practical applications of residues analysis of difficult pesticides and to transfer knowledge on the principles of method validation, separation science, method optimization, quality control and quality assurance.

*Training workshop on 'Data Quality for Decision Making'*

The FEPL, in collaboration with the Centro en Contaminacion Ambiental (CICA), an IAEA collaborating centre, held a regional training workshop on 'Data Quality for Decision Making' in San José, Costa Rica, on 18-19 May 2017, immediately after the sixth Latin American Pesticide Residue Workshop. The workshop was funded under TCP RLA7019, "Developing indicators to determine the effect of pesticides, heavy metals and emerging contaminants on continental aquatic ecosystems important to agriculture and agroindustry", and targeted statistical strategies for the generation of accurate and reliable pesticide residue results for decision making processes, and provided a forum for interdisciplinary networking between stakeholders in the farm-to-fork food chain.



The workshop was attended by 44 participants from 12 countries (Argentina, Brazil, Chile, Colombia, Costa Rica,

Guatemala, Mexico, Nicaragua, Panama, Paraguay, Spain and Uruguay).

## RALACA Laboratory Network

The Red Analítica de Latino America y el Caribe (RALACA) is a non-profit regional network of laboratories and associated institutions in Latin America and Caribbean countries, initiated under an FEPL project, that aims to enhance regional capabilities to target food safety and environmental sustainability (http://red-ralaca.net). The Joint FAO/IAEA Division is represented on the Board of RALACA by Ms Britt Maestroni (FEPL). RALACA held its second general meeting in May 2017, when two new institutes enrolled, bringing membership to 54 institutions in 21 countries. The visibility and popularity of the network is constantly improving, with the number of guests on the RALACA website reaching more than 1000 per day.

Interaction of FEPL with RALACA in 2017 produced a number of outputs, included six scientific webinars, six leaflets, seventeen posters, one online module on data management and a newsletter article.

## Field Missions

In addition to the training courses and workshops, FEPL staff carried out several missions to Member States to provide technical assistance, policy advice and project planning.

**National outreach seminar on United Arab Emirates' Technical Cooperation with IAEA**

A national outreach seminar on United Arab Emirates' Technical Cooperation with IAEA was held at Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates, 20-22 August 2017. Discussions focused on areas of nuclear applications where IAEA can provide support through its TC Programme to address the Sustainable Development Goals (SDGs) and national needs. The audience included existing stakeholders of UAE's TC Programme with IAEA and new stakeholders in all focus areas, including governmental entities and ministries, food and agriculture, environment, energy, health and industrial applications. Presentations were made by staff representing several IAEA departments and programmes, including Mr Simon Kelly, representing the Joint FAO/IAEA Programme.

**Implementing the Stable Isotope Technique for High Quality Agro-product Traceability and Authenticity in China**

Verifying food authenticity is a high priority for China; not only to protect consumers from fraud, but also to protect them from unintended food safety issues that are derived from clandestine food production activities in unlicensed or unsanitary conditions. The Technical Cooperation Project CPR5022 on 'Implementing the Stable Isotope Technique for High Quality Agro-product Traceability and Authenticity' aims to provide a solution to the problem of inferior agro-products being passed off as high-quality products in the Chinese domestic and export markets. The FEPL has been working with the Chinese Academy of Agricultural Sciences' (CAAS) Institute of Quality Standards and Testing Technology for Agro-Products (IQSTAP) to develop systems utilising advanced stable isotope techniques and an integrated and multidisciplinary approach to characterise the identity and authenticity of Chinese agro-products - specifically pork, rice and milk.

In March 2017, Mr Simon Kelly (FEPL) visited IQSTAP to discuss and review the planned project activities and the purchase and installation of a bench top nuclear magnetic resonance spectrometer for species identification in meat products.

**Safety of Food of Animal Origin in Sri Lanka**

Mr Andrew Cannavan travelled to Sri Lanka to advise the relevant authorities and counterparts and facilitate the development of a new technical cooperation project design on the safety of animal originated food, to commence in the 2018-2019 TC cycle. Mr Cannavan visited the two counterpart institutes for the project, the Sri Lanka Atomic Energy Board (SLAEB) Laboratories in Colombo and the

Faculty of Veterinary Medicine and Animal Science in Peradeniya University. The objectives of the project were discussed in the framework of food safety problems encountered in Sri Lanka, especially with respect to chronic kidney disease, which has unknown etiology. A major problem is the current lack of baseline data that could be used to support the development of regulations and enforcement actions to enact food safety legislation. At Peradeniya, discussions focused on the necessity to expand the testing capabilities developed under previous TCPs for veterinary drug residues and other chemical contaminants in food. Mr Cannavan also visited the Sri Lankan Tea Research Institute at Talawakelle to investigate possible synergy with the SLAEB laboratories, and to discuss the development of a TCP on authenticity testing of tea.



### *Advice and Information Exchange*

In addition to the technical and policy advice provided through technical cooperation projects and the RALACA network, the FEPL interacted extensively with Member States to provide advice and information. Examples include the provision of technical advice to the **Seychelles** Public Health Laboratory on validation of a screening test for aflatoxin M1 in dairy products, to the Quality Control Council (QCC) of the **United Arab Emirates** on confirmation of authenticity of honey and to **Sri Lanka** on authenticity testing and adulteration detection in tea.

FEPL staff regularly provide advice through participation as members of, for example, the UK's Food Authenticity Methodology Working Group, the advisory board of the ASSET (Assured, Safe and Traceable Food) Centre at Queen's University Belfast, UK, the Global Food Safety Partnership, and the International Food Safety Network (INFOSAN).

The FEPL participated in an informal debate on edible insects, organised by the United Nations Information Service, with the screening of the film "Bugs", sponsored by the Permanent Mission of Denmark to the United Nations in Vienna. The debate discussed the potential and challenges of edible insects as a possible source of proteins to respond to the growing demand for feed and food security. A consequent follow-up meeting and discussion was held with the Austrian Development Agency.

Staff of the FEPL are represented on the scientific or organising committees of three upcoming international conferences: the Eighth International Symposium on Hormone and Veterinary Drug Residue Analysis, Ghent, Belgium, 22-25 May 2018; the Belfast Summit on Global Food Integrity, Belfast, UK, 28-31 May 2018; and IUPAC 2019 - 14th International Congress of Crop Protection Chemistry, Ghent, Belgium, 19-24 May 2019.

The FEPL was included in the evaluation panel for designation of FAO Reference Centres for Antimicrobial Resistance. Twenty-six applications were evaluated and recommendations made to assist in decision making by FAO Headquarters.

### Fellowships, Scientific Visitors and Interns

In April 2017, Mr Amer Abu Alnasser completed a 3-month fellowship in FEPL under TCP SYR5023, Enhancing Analytical Capacities of Major Pesticide Residues, which aims to improve the scope and quality of pesticide residue analysis of food and food products in Syria to enhance monitoring, food safety and comply with trade standards. Mr Alnasser worked on the development and validation of a multi-residue method for the detection and quantification of a range of selected pesticides in vine leaves, using gas chromatography – tandem mass spectrometry.

Under the same project, Mr Iyad Ghanem, from the Atomic Energy Commission of Syria, completed a scientific visit of one week in the FEPL in October 2017. Mr Ghanem's programme focused on the

analysis of contaminated vine leaves using the method validated during Mr Alnasser's fellowship, and included data analysis and interpretation. The study is expected to lead, in the near future, to a publication on the dissipation of selected pesticides on vine leaves.

Ms Amber Vaughan joined the FEPL team in late September 2017 for a one-year internship focusing on food sample analysis using isotope ratio mass spectrometry (IRMS). Ms Vaughan graduated with a Master's degree in Earth Science from the University of East Anglia, UK, in summer 2017 and brings experience and knowledge of stable isotope measurements in a geochemistry context to FEPL. Her internship will enable her to broaden her experience through hands-on research into stable isotope measurements for food authenticity and to support food traceability systems, and to contribute to the FEPL outputs in this field.

Ms Valentina Centonze joined the FEPL at the start of October for a 3-month internship from the Department of Chemistry of the University of Bari "Aldo Moro" in Italy. Her PhD studies focus on the development of innovative methods for food quality and safety. Ms Centonze's work during her internship focused on the discrimination of oranges by variety and geographical origin using spectroscopic and mass spectrometric molecular fingerprinting techniques and chemometrics.

Within the framework of ongoing scientific collaboration with the Faculty of Chemistry, Universidad de la Republica, Montevideo, Uruguay, Ms Veronica Cesio visited the FEPL for two weeks in November 2017 to complete some joint studies on pesticide residue analysis. The studies focused on the use of analyte protectants to improve analytical method performance and processing factors for herbs and infusions of herbs, and the corresponding validation criteria.

Ms Brenda Checa, from the National Plant Protection Section of the Ministry of Agriculture in Panama, completed a scientific visit of two weeks in November 2017, under TCP PAN5024, Developing Analytical Capabilities for the Detection of Chemical Contaminants in Food and the Quality of Agrochemicals. Ms Checa's programme focused on the optimization of gas chromatography coupled to mass spectrometry to confirm positive results found using screening techniques such as electrochemical biosensors and ELISA, as applied in Panama.

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## EXTRA-BUDGETARY SUPPORT

# THE INSECT PEST CONTROL LABORATORY

# EXECUTIVE SUMMARY

In the Livestock Pest (LP) group of the Insect Pest Control Laboratory (IPCL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, work continued to assess the feasibility of exploiting potential beneficial traits conferred to tsetse flies by their gut microbiota in an attempt to improve the rearing of these insects. Gut microbiota species were identified and their dynamics with the ageing of the flies assessed. Furthermore, work was initiated to assess the effect of radiation on these microbiota. Initial results indicate that the densities of the bacteria were negatively impacted by radiation.

Studies were also carried out to assess the infection levels of the *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV) in natural populations of different tsetse fly species. The study indicated that GpSGHV infects at least seven *Glossina* species, but the distribution, diversity and prevalence of the virus was highest in *G. pallidipes*. It therefore appears that GpSGHV has evolutionarily reached a stable but dynamic equilibrium in all tested *Glossina* species other than *G. pallidipes*, which may account for SGH outbreaks in this tsetse species.

Initial data using a near-infrared sorter have indicated a possibility to separate male from female tsetse pupae on day 22 post-larviposition. Therefore, the effects of radiation and transport were assessed on male *Glossina morsitans morsitans* that were irradiated during the pupal phase on day 22 post-larviposition and transported to Antwerp, Belgium. These flies had a significantly lower survival rate than non-transported and non-irradiated flies. These results will have implications for long-distance shipments of pupae that will have received the treatment.

In the Plant Pest (PP) group, work continued under the FAO/IAEA/USDA agreement on Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies. In one study, populations of the oriental fruit fly, *Bactrocera dorsalis*, from China, Kenya and Thailand were not significantly different in tolerance to vapour heat treatment at 48°C. The results allowed for broadly applicable vapour heat treatments to be adopted by the International Plant Protection Convention.

More work was done on the combined use of the male annihilation technique (using a male attractant [methyl eugenol (ME)] and insecticide in baits) and the sterile insect technique (SIT) for *B. dorsalis*. It was previously reported that ME-airblown-aromatherapy provided to 14-15-day-old *B. dorsalis* males enhanced their mating competitiveness. Follow-up research focussed on assessing whether the sexual maturation of the males could be shortened and mating competitiveness improved through adding protein supplements, a juvenile hormone analogue or ME. Adding protein supplements significantly increased the mating success of the males, with more than 50% of the protein-fed males achieving a mating as of day 5. Five to 7-day-old protein-fed *B. dorsalis* males treated by ME-airblown-aromatherapy achieved significantly more successful matings as compared with 14-day-old untreated males in walk-in field cages.

A genetic sexing strain of the South American fruit fly, *Anastrepha fraterculus*, (based on a colour mutation of the pupa) was characterized with respect to its biological features, its mass production and quality profile and its genetic stability. Conversely, an introgression approach was adopted to develop a white-pupae strain for *B. tryoni* with the white pupae (*wp*) gene of *B. dorsalis* as target marker. Laboratory mating compatibility tests in small cages indicated sexual compatibility between the hybrid *B. tryoni* males and *B. tryoni* wild-type females. Finally, radiation dose-response curves were developed for *Drosophila suzukii* and important progress was made with the development of ovipositioning substrates to enhance productivity of *D. suzukii* colonies.

In the Human Disease Vectors (HDV) group of IPCL, research focussed on the development of cheaper larval diets for *Anopheles arabiensis*, *Aedes aegypti* and *Aedes albopictus*. The focus was on finding a cheaper alternative to replace the very expensive bovine liver powder in the larval diet. Assessments

were carried out with chickpea or insect proteins derived from black soldier flies. They were validated for the main entomological parameters (development time, survival rate from L1 to pupae and adults, egg production and flight ability) and initial results indicate that the cost of the larval diet can indeed be reduced by more than 50%.

Two new quality control devices were developed and successfully tested to measure mosquito flight ability, i.e. an aspirator device that imposes an aspiration stress on the mosquitoes, and a simple flight organ device.

A first prototype of a sterile male mosquito release device carried by a drone was tested indoors at the IPCL and the impact on damage, longevity and flight ability of male mosquitoes tested.

In the Genetics and Molecular Biology (GMB) group of the IPCL, the complete polytene chromosome map and mitotic karyotype of *Ceratitis fasciventris*, a member of the *Ceratitis* FAR complex, was published, and molecular, genetic and cytogenetic analysis of two additional members of the complex (*C. rosa* and *C. quilici*) is ongoing.

Mitotic and polytene chromosome analysis is ongoing in two *Bactrocera* species (*B. correcta* and *B. zonata*) and one *Zeugodaucus* species (*Z. tau*) and progress can be reported with the cytogenetic analysis of *D. suzukii*. The polytene chromosome map of this species is under construction, while preliminary *in situ* hybridization experiments on polytene chromosomes gave promising results.

The life history traits of naturally *Wolbachia*-infected lines of *D. suzukii* and of *Wolbachia*-free lines, which were produced by antibiotic treatments, were assessed. This initial comparative analysis has provided evidence that *Wolbachia* infection has a cost, a finding that needs to be taken into consideration in deliberating whether an infected or an uninfected line should be used for the mass-rearing and potential SIT applications against this major pest.

In 2017, staff and/or visiting scientists of the IPCL published 26 articles in peer reviewed journals.

In 2017, the IPCL hosted 12 cost-free experts and 12 consultants (of which five were PhD students), six interns, ten fellows and one scientific visitor.

The GMB/PP groups carried out 47 fruit fly shipments to 20 institutions in Austria, Bangladesh, France, Germany, Greece, Guatemala, Italy, Mexico, Panama, Senegal, Spain and Sweden, and six shipments of preserved fruit flies to six institutions in Benin, Brazil, China, Italy, the Netherlands and the USA. The LP group carried out 177 shipments of tsetse pupae (a total of 372 543 pupae of which 239 744 *G. palpalis gambiensis* were shipped to Senegal) to ten different institutions in Belgium, Burkina Faso, France, Greece, Senegal, South Africa, Uganda, the UK and the USA. The HDV group carried out 25 shipments of mosquitoes to nine institutions in French Polynesia, Germany, Italy, Spain, Sweden and Switzerland.

In 2017, the IPCL received 459 official visitors from 75 countries.

## STAFF

| Name | Title |
|---|---|
| **Vreysen**, Marc | Laboratory Head |
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| **Bourtzis**, Kostas | Molecular Biologist/Geneticist |
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| **Caceres**, Carlos | Entomologist (Plant Pests) |
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# MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

## Livestock pests

### *Identification of cultivable tsetse gut microbiota and its effects on fly performance*

As stated in the activity report of 2016, controlling tsetse flies remains the most effective and sustainable strategy for controlling African trypanosomoses (nagana in cattle and human sleeping sickness in humans). The SIT has great potential when implemented within area-wide integrated pest management (AW-IPM) approaches against this pest. The SIT requires mass-production of high-quality sterile males. It is known that insect microbiota can improve the mass-rearing of insects when used as a probiotic. Therefore, it was important to assess the potential beneficial traits conferred by the tsetse gut microbiota and its potential to improve tsetse mass-rearing. We used culture-dependent methods to multiply cultivable bacteria and to assess their probiotic potential. Notable of the identified gut microbiota were members of the families Microbacteriaceae (genus *Microbacterium*), Sphingobacteriaceae (genus *Sphingobacterium*) and Moraxellaceae (genus *Acinetobacter*). These bacterial species have been reported in other insects, some of which are documented to confer beneficial traits to their insect hosts. These results indicate that the densities of the cultivable gut microbiota significantly decreased in 10-day-old flies as compared to 6-day-old flies; this age is important for the release of sterile males in SIT programmes.
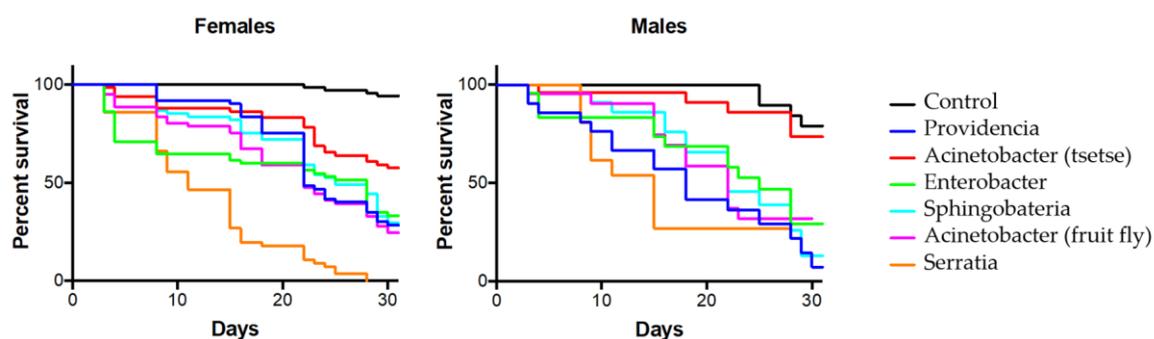


FIG. 1: Impact of gut microbiota used as probiotic on tsetse fly survival

Secondly, the gut microbiota community was composed of both beneficial and potentially harmful bacterial species. The attempts to use some of the cultivable bacterial species as probiotics resulted in a significant increase in fly mortality (Fig. 1), indicating the need for further research to amend this approach. This increased mortality might indicate the presence of harmful bacterial species in the gut microbiota that might require further analysis and mitigating measures.

In addition, we used culture-independent methods (new generation sequence of 16S rRNA gene) to assess the microbiota diversity and to assess the impact of radiation on gut microbiota. The preliminary results indicate that the radiation treatment significantly reduced tsetse gut microbiota (Fig. 2).



FIG. 2: Impact of radiation treatment of tsetse gut microbiota

## Molecular identification of tsetse species

The objective of this study was to develop quick, cheap and easily applied tools to identify tsetse sub-species and different haplotypes within the same sub-species in Africa for SIT application. In the activity report of 2016 we presented molecular identification tools based on a combined approach using different molecular markers, i.e. a nuclear marker (internal transcribed spacer 1 (IT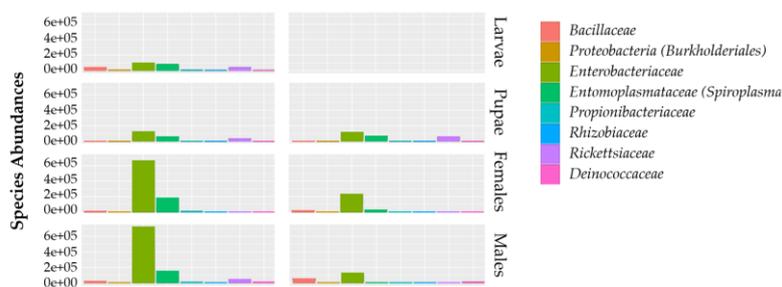S1)), microsatellite markers, mitochondrial markers (COI, COII, 12S and 16S rRNA) and *Wolbachia* 16S rRNA. Although this tool was effective to discriminate amongst tsetse species, it remains a laborious and costly method as it needs several steps and DNA sequencing. Therefore, a new method based on mitochondrial DNA was developed to distinguish between the tsetse sub-species and between haplotypes/populations of the same tsetse species from different geographical locations. In this study, the mtDNA genomes of seven tsetse species: *G. pallidipes*, *G. m. morsitans*, *G. m. centralis*, *G. p. gambiensis*, *G. f. fuscipes*, *G. austeni* and *G. brevipalpis,* were sequenced, followed by a comparative analysis of the mtDNA of the seven species to identify a variable region that can be used to distinguish the species. High resolution melt (HRM), a technique that applies post-PCR analysis of the melt curve to identify genetic variation of nucleotide sequences, was applied on the tsetse mtDNA variable region to differentiate tsetse spp. and haplotypes/populations within the species (Fig. 3).
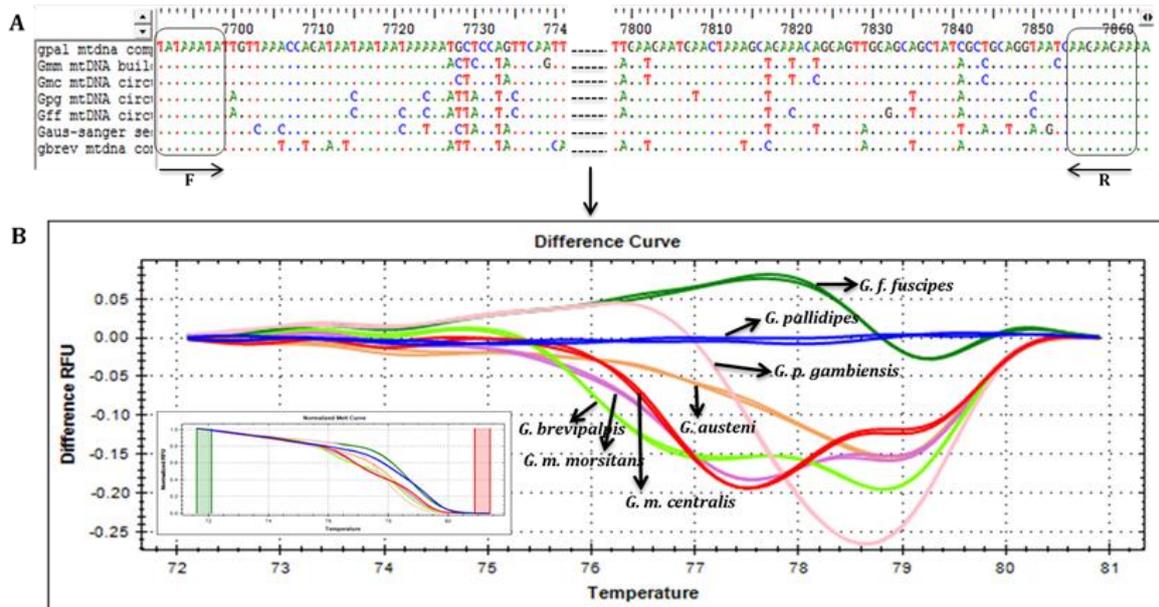


*FIG. 3: Mitochondrial genome analysis of seven tsetse species. (A) An example of a variable region among the species mtDNA and (B) the application of HRM to distinguish the tsetse species*

## Prevalence and genetic diversity of SGHV in wild tsetse species

Tsetse flies are naturally infected by the *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV), a pathogenic virus specific to some *Glossina* spp. All the *Glossina* species screened so far have shown different infection levels with SGHV, both under field and laboratory conditions. The objective of this study was to determine the prevalence and genetic diversity of the virus in different wild tsetse species and populations. Tsetse fly species analysed in this study were sampled in different geographical locations in eastern and southern African countries. These included *G. pallidipes*, *G. morsitans morsitans*, *G. fuscipes fuscipes*, *G. palpalis palpalis*, *G. brevipalpis* and *G. austeni*. Five candidate genes were selected for this study (orf9, orf10, orf38, VNTR-R1 and VNTR-R2).

The maximum likelihood phylogenetic tree using the five genes illustrated that although GpSGHV infects at least seven *Glossina* species, the distribution, diversity and prevalence of the virus was highest in *G. pallidipes*. It therefore appears that GpSGHV has evolutionarily reached a stable but dynamic equilibrium in all tested *Glossina* species other than *G. pallidipes*, which may account for SGH outbreaks in this tsetse species. These results imply that *G. pallidipes* is the major contributing species for evolution and interspecies spread of GpSGHV in tsetse mass-rearing facilities, and therefore care must be taken in the handling of this tsetse species in facilities that rear multiple tsetse species (Fig. 4).
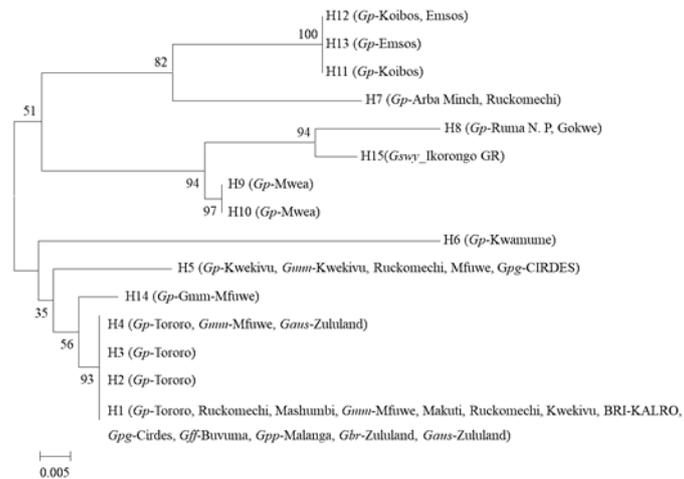


*FIG 4: Phylogenetic tree resulting from the maximum likelihood method based on concatenated sequences of orf9, orf10, orf38 VNTR_R1 and VNTR_R2 sequences. The bootstrap values are also indicated based on 1000 replicates*

### *Effect of transport and irradiation on the longevity of* Glossina morsitans morsitans *males*

The implementation of the SIT requires the production of sterile males of adequate biological quality. The released sterile males should survive long enough in the field to be able to compete with wild males for mating with wild females. In certain SIT programmes, the mass-rearing facilities are located at a significant distance from the release area, which requires the aerial transport of irradiated males. In past and current tsetse programmes that have an SIT component, the males are irradiated as adults, according to the view that separating females from males is still not possible at the pupal stage (and females need to be retained for production reasons). Recent attempts to separate sexes at the pupal stage using a near-infrared sorter device indicate the potential of separating male from female pupae on day 22 post-larviposition. Therefore, we assessed the effect of radiation treatment combined with transport of pupae on day 22 post-larviposition on male survival. The results indicate that male *G. m. morsitans* that emerged from pupae exposed to 110 Gy and transported as pupae from the IPCL in Seibersdorf, Austria to the Institute of Tropical Medicine in Antwerp, Belgium had a significantly lower survival rate as compared with non-transported
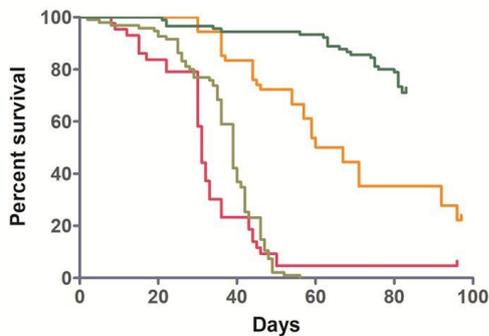


*FIG 5: Impact of irradiation (110 Gy) and transportation of G. m. morsitans male pupae on adult survival. Red line: transported and irradiated male pupae. Light green line: non-transported irradiated. Orange line: transported non-irradiated. Dark green line: non-transported non-irradiated*

and non-irradiated males (Fig. 5).

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## Plant pests

### *Phytosanitary treatments under the FAO/IAEA/USDA agreement*

The development of phytosanitary irradiation, heat and cold treatments has continued under the FAO/IAEA/USDA agreement on "Development of Phytosanitary and Regulatory Treatments for Exotic Tephritid Fruit Flies". This agreement aims to develop broadly applicable phytosanitary treatments against fruit-infesting tephritids in collaboration with researchers and institutions worldwide. The availability of diverse fruit fly colonies/strains/species at the IPCL makes it an ideal place to develop broadly applicable, proactive phytosanitary treatments.

The results generated by the phytosanitary treatment research carried out at the IPCL can be used as a guideline to develop phytosanitary treatments schedules in FAO and IAEA Member States. Ultimately, besides supporting activities and guiding phytosanitary treatments recommended by the International Plant Protection Convention (IPPC), our results can facilitate trade among Member States through the application of phytosanitary treatments that improve quarantine security.

Prior studies focusing on defining target irradiation doses against fruit flies have significantly contributed to advancing the use of phytosanitary irradiation worldwide. However, more research needs to be done to determine whether low-oxygen atmosphere conditioning, a treatment widely applied to preserve commodity quality, increases the insect's radiotolerance and reduces the efficacy of irradiation treatments. Research on the influence of low-oxygen atmosphere on phytosanitary irradiation efficacy against *Anastrepha ludens*, *Bactrocera dorsalis*, and *Ceratitis capitata* is currently addressed at the IPCL. Our research intends to assess the potential risk of increasing insect survival in infested commodities treated with phytosanitary irradiation under low-oxygen conditions. The results of this study can contribute to the revision of restrictions applied by regulatory agencies to phytosanitary irradiation under a modified atmosphere.



FIG. 6: *Ms Amanda Amorim Da Silva Cardoso from Brazil and Mr Camilo Rivera from Guatemala dissecting fruits for phytosanitary treatment research*

In another study, oriental fruit fly *Bactrocera dorsalis* populations from China, Kenya and Thailand were not significantly different in tolerance to vapour heat treatment at 48°C. The results allowed for broadly applicable vapour heat treatments to be approved by the IPPC, have been written up for publication and are currently being reviewed by the Florida Entomologist.

Another study addressed cold treatments required to export fresh fruit from subtropical areas of Asia, where many fruit fly species exist for which there are no cold treatments. Two different endpoints have been used by plant protection organizations to develop cold treatment schedules: 1) complete mortality of all fruit fly eggs and larvae present in the commodity treated or 2) prevention of subsequent pupation by any larvae initially surviving the treatment. The latter is usually achieved with less time than the former; however, it leaves a small and probably insignificant possibility that live larvae could be found upon inspection, which may lead to rejection of the lot of fruit. Research with *Bactrocera (Zeugodacus) tau* found the 3rd instar to be the most cold-tolerant stage. When treating infested mandarins at 1.0-1.2°C, mortality of all 3rd instar larvae was obtained after 22 days and prevention of pupation after 20 days.

### Reviewing irradiation protocols for Sterile Insect Technique application - Overexpression of antioxidant enzymes and the effect of low-oxygen atmosphere treatment and irradiation on the quality of Caribbean fruit fly males

Ms Vanessa Dias de Castro from Brazil has been collaborating with US scientists and IPCL staff to assess the effects of a key antioxidant enzyme on the sexual performance and quality of sterile male Caribbean fruit fly *Anastrepha suspensa*, treated (or not) under a low-oxygen atmosphere before and during gamma irradiation. In operational fruit fly programmes that have an SIT component, late pupae are sterilized under a low-oxygen atmosphere to minimize the adverse effects of ionizing radiation. This pupal pre-irradiation treatment with a low-oxygen atmosphere can increase enzymatic antioxidant capacity, reduce oxidative damage and improve sterile insect performance, as shown by previous correlative studies.
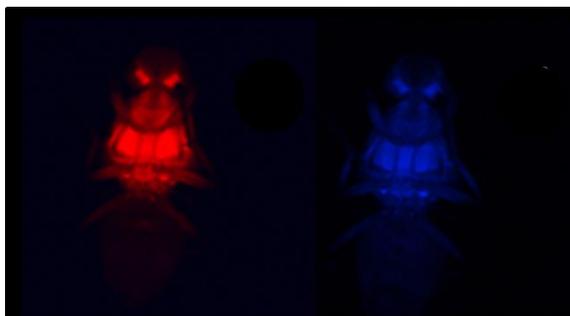


FIG. 7: Gene expression of the fluorescent protein markers, DsRed (red) and AmCyan (blue), in transgenic Caribbean fruit flies

In our study, we tested the effect of antioxidant enzyme overexpression on sexual performance and quality of sterile *A. suspensa* males. Transgenic males (Fig. 7) that overexpress the mitochondrial superoxide dismutase (SOD2) were compared with wild-type males in multiple tests to evaluate whether increasing antioxidant capacity can decrease oxidative stress, increase mating success and improve flight propensity after irradiation. Briefly, we compared the total antioxidant capacity, sexual performance, flight propensity, and sterility of wild-type and transgenic males overexpressing SOD2 (SOD2 5.2), irradiated under normoxia (normal air), and two low-oxygen atmosphere regimes (hypoxia and quasi-anoxia). Despite being 10% more sexually successful than the wild-type flies irradiated under normoxia, overexpression of SOD2 did not synergistically improve the mating success of males irradiated in hypoxia or quasi-anoxia.

Hypoxia and quasi-anoxia, *per se*, increased the total antioxidant capacity and improved the sexual competitiveness of the wild-type males. Lek positioning of normoxia-irradiated insects was affected in comparison to non-irradiated insects but did not differ for both hypoxia- and quasi-anoxia-irradiated males. Also, males irradiated in hypoxia and quasi-anoxia remained sterile, similarly to males irradiated in normoxia. The results from this study allow a better understanding of the biological effects of radiation in sterile insects and reinforce the use of pre-irradiation treatments that protect flies against the side-effects of ionizing radiation.

### Semiochemicals, food supplements and hormone analogues to improve the sexual competitiveness of sterile Oriental fruit fly males

Feeding on methyl eugenol (ME) is known to enhance the mating competitiveness of *Bactrocera dorsalis* males, which can substantially increase the cost effectiveness and biological efficiency of the SIT. However, supplementing ME through feeding is not practical in sterile male production facilities, so a practical method of ME application is urgently required. Previously we reported that ME-airblown-aromatherapy provided to 14-15-day-old *B. dorsalis* males enhanced their mating competitiveness and this method of ME application would be of practical use in sterile male production facilities. However, holding sterile male *B. dorsalis* for two weeks until they attain sexual maturity and respond to ME would not be very cost-effective. Therefore, studies were conducted to assess whether adding food supplements, a juvenile hormone analogue or a semiochemical (ME) as an independent treatment or in combination could accelerate the sexual maturation in *B. dorsalis* males and enhance their mating competitiveness. The age of peak sexual maturity of male *B. dorsalis* of a genetic sexing strain (GSS) was determined and followed by an assessment of whether these different treatments could accelerate the sexual maturation of the males. In addition, the effect of

ME-airblown-aromatherapy on the mating competitiveness of young males as compared with sexually mature males was also assessed.

The results showed that protein supplements significantly increased the mating success of the males, with more than 50% of the protein-fed males achieving a mating as of day 5. Males fed on only sugar needed more than 14 days to achieve a similar level of mating success. Topical application of a juvenile hormone analogue alone or in combination with ME feeding had no additional advantage over protein-fed males and the precocious period (5 days for protein fed males) was not further reduced. Five to 7-day-old protein-fed *B. dorsalis* GSS males treated by ME-airblown-aromatherapy achieved significantly more successful matings as compared with 14-day-old untreated males in walk-in field cages.

## The South American fruit fly

As reported previously, Mr Salvador Meza and Ms Martha Guillen from Mexico, together with Ms Silvana Caravantes from Guatemala have been characterising a GSS of the South American fruit fly *Anastrepha fraterculus* that is based on a pupal colour dimorphism (brown-black) (Fig. 8). The mutation is the result of a reciprocal translocation between the Y chromosome and the autosome carrying the wild-type locus of the black pupae (*bp*) gene. The GSS was constructed from a laboratory population of the "aff1-morphotype", which implies that the SIT with male-only releases could in theory be applied over a large target area that extends from southern Brazil to central Argentina. Four GSS have been evaluated to determine their biological characteristics, their mass-production and quality profile and their genetic stability. Results of this evaluation will allow the selection of the best potential candidate for male-only field releases. Screening of more translocated lines are ongoing for further analyses.



FIG. 8: (Left) wild type male pupae (brown) and (right) black female pupae

## Drosophila suzukii

As reported previously, the IPCL has been an active counterpart in the SUZUKILL project, funded by the French Agence Nationale de la Recherche and the Austrian Wissenschaftsfonds. One of the objectives of this project is to develop alternative and innovative approaches for the biological control of *Drosophila suzukii*, a serious invasive pest. The objective of the IPCL is to develop the "SIT package" for this pest and to assess the feasibility of its use in confined areas such as greenhouses.

To be able to implement the SIT for this pest, studies on irradiation/biology of the target insect is required. More specifically, the optimal dose that sterilizes the males without compromising the quality and performance of the released sterile insects in the field is needed. Recently we have completed the dose-response studies for both male and female *D. suzukii*.

Good progress has been made with the development of an oviposition system that is composed of an oviposition panel made of netting that simulates the oviposition system used to mass-rear other fruit fly species of economic importance (Fig. 9). A practical oviposition system is critical for the production of large numbers of eggs. The current system allows the eggs to be washed from the oviposition panel, easily cleaned, quantified and incubated. The success of this research will contribute to the development of environment-friendly and practical solutions to better manage the threat of *D. suzukii*. Further work is ongoing to fine-tune the system into an optimal economic mass-rearing system. Production of a larger
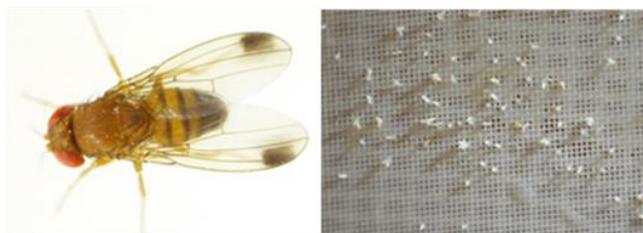


FIG. 9: (Left) Adult Drosophila suzukii *and (right) oviposition panel (made of netting) and showing the oviposited eggs*

number of pupae will also allow further work on irradiation dose response curves and irradiation protocols.

### Bactrocera tryoni *white pupae hybrid strain*

Ms Mitzy Porras from Colombia worked at the IPCL on an assessment of quality control parameters of the white pupae hybrid strain of *Bactrocera tryoni*. *B. tryoni* is a major pest of fruits in Australia, and key insecticides traditionally used to manage the pest have recently been restricted, resulting in increased demands to develop environment-friendly control methods such as the SIT. Recently, SIT managers in Australia have shown strong interest in the development of a GSS for this fruit fly to enable male-only releases to enhance SIT efficiency.

Currently, no morphological markers are available for *B. tryoni* that would allow the development of a practical GSS. It was therefore suggested to adopt an introgression approach, creating a white-pupae line of *B. tryoni* with the white pupae (*wp*) gene of *B. dorsalis* as target marker. The scheme of introgression of the *wp* gene into the genome of *B. tryoni* has involved several crosses and backcrosses between males of *B. tryoni* and white-pupae females of the *B. dorsalis* line (Fig. 10).

First results of laboratory mating compatibility tests in small cages have shown sexual compatibility between the hybrid *B. tryoni* males and *B. tryoni* wild-type females. The promising results of the present study encourage further investigations



FIG. 10: (Left) Bactrocera tryoni *wild type pupa, and (right) white pupae hybrid strain*

using walk-in field cages and assessments of chemical signals that might drive the compatibility between the hybrid strain and wild *B. tryoni* females.

### *Potential use of inactive bacteria as a protein source for the mass-production of fruit flies of economic importance*

Studies on the development of new larval diets for the mass-rearing of Mediterranean fruit fly indicated that using inactive bacteria of the genus *Enterobacter sp*. as a replacement for brewer's yeast increased larval production and the quality of the larvae, pupae and the adults. These studies clearly demonstrated that the quality of the protein originating from bacteria is equal or superior to that of the protein present in brewer's yeast. Further benefit-cost analyses will be carried out to determine the viability of this approach on an industrial scale.

## Human disease vectors

### *Development of optimal larval diets to mass-rear* Anopheles arabiensis, Aedes aegypti *and* Aedes albopictus

Traditional larval diets to rear *Anopheles arabiensis*, *Aedes aegypti* and *Aedes albopictus* are based on bovine liver powder (BLP), tuna meal (TM) and brewer's yeast (BY). These diets are all rather expensive and the ingredients not always available in Member States, which compromises the efficiency and sustainability of the SIT for mosquitoes. The BLP is by far the most expensive component, costing more than 78 times the price of TM, and more than 6 times the price of BY.

Therefore, efforts have been undertaken to improve the cost-efficacy of these diets. Four diet mixtures, including TM, BY and chickpea (CP) and 0-25% BLP, are now available for *An. arabiensis* rearing, which entails a 40-92% reduction in costs. Furthermore, investigations into using bacteria dry mass as a nutritional source improved the diets for the rearing of *Anopheles* and *Aedes* species.

Finally, effective insect-based larval diets, including insect proteins without the addition of BLP, have been developed for *An. arabiensis*, *Ae. aegypti* and *Ae. albopictus*. New diets that include insect

proteins derived from black soldier flies have been tested with success for *An. arabiensis*, *Ae. aegypti* and *Ae. albopictus* on a small laboratory scale and for *Ae. aegypti* under mass-rearing conditions. They were validated for the main entomological parameters (development time, survival rate from L1 to pupae and adults, egg production and flight ability) and might allow reduction of the cost of larval diet by more than 50%.

### *Quality control methods for mosquito SIT*

The ability of released sterile males to survive, disperse, compete with wild males and inseminate wild females is an essential prerequisite for success in a mosquito suppression programme that includes an SIT component.

Adequate quality control tests using standardized procedures need to be developed and adopted to identify problems and enable the adjustment of the rearing, handling and release methods to ensure optimal male quality.

The ability of insects to fly is one of the most direct and reliable indicators of insect quality; the ability of an insect to fly out from a simple flight tube is a standard quality control procedure routinely used in many operational SIT programmes for fruit flies and tsetse flies.

Two novel quality control devices have been developed and designed to measure, compare and infer the sterile male mosquito competitiveness by observing their flight ability (Fig. 11). Survival and insemination potential of irradiated males were successfully predicted using their ability to fly out of narrow plastic tubes (flight organ device) or after aspiration stress exposure (aspirator device).

These simple and cheap tools are practical instruments to identify and correct sub-optimal rearing and handling procedures and to evaluate single and cumulative stress during male production, which may affect the final male quality and their field performance.

The availability of easy and standardized quality control tests could facilitate the adoption of international procedures for evaluation and comparison of mosquito strains and to assess strain suitability for effective mosquito genetic control applications.



FIG. 11: Various systems developed to measure mosquito flight ability. Top, aspirator device used to impose an aspiration stress exposure to mosquitoes; bottom left, 40 cm high flight organ device used to test the quality of adults emerging from pupae; bottom right, flight organ device used to test the quality directly of adults

### *Developing a drone for aerial release of sterile male* Aedes *mosquitoes*

Towards the end of 2016, IPC colleagues, together with the NGO WeRobotics, successfully secured an award of US $400 000 from the United States Agency for International Development (USAID) under a call for "Combatting Zika & Future Threats: A Grand Challenge for Development". Our proposal, entitled "Fighting Future Threats Using Autonomous Aerial Robotics", focussed on designing a mechanism to be deployed with an unmanned aerial vehicle (UAV) to release sterile male mosquitoes.

The aim is to develop a continuous release system, which can release around 100 000 mosquitoes during a 10-minute flight. To design the holding container, we first needed to determine the maximum column height or highest tolerable level of compaction that could be imposed on immobile mosquitoes. This was calculated by compacting several thousand immobile mosquitoes inside prisms designed to replicate a prototype holding container and then exposing them to varying weights of

particles above them, intended to replicate different quantities or column heights of mosquitoes. Each prism was then held at 8°C for one hour inside an incubator, and thereafter, mosquitoes were split into two cages, depending on whether they had been in the top or bottom layer whilst compacted. A small sample was taken from both layers for all prisms and mosquitoes were screened under a stereomicroscope for damaged or missing wings and legs.

Additionally, longevity was assessed in each cage until all mosquitoes had died. From experiments performed thus far at the IPCL, the procedure of immobilization did not appear to impact subsequent survival. When held immobile for up to 24 hours at 4-10°C, although slightly reduced, survival was not significantly lower than that of controls.

In March 2017, the first prototype of the release device was tested in the greenhouse of the IPCL and



*FIG. 12: Field test of a drone equipped with a mosquito release device*

the impact on damage, longevity and flight ability of male mosquitoes tested. Based on the results of these tests, further modifications were made to the release device. With the help of the IAEA's Nuclear Science and Instrumentation Laboratory team, we designed and 3D printed a release device based on existing systems used for aerial release of other sterile insects in the framework of the European Research Council's REVOLINC project (PCT/EP2017/059832). After investigating flowability (homogeneity of the release rate), the most suitable prototype was selected. The use of the unmanned aerial vehicle and release system did not impact the quality of sterile male *Ae. aegypti* measured as flight ability (Fig. 12).

The impact of various wind speeds was also investigated to determine the maximum flying speed of the drone. A wind tunnel has been built especially for this test to simulate similar conditions that mosquitoes will encounter upon being ejected from the release system. Batches of male mosquitoes were compacted and held immobile for various durations and flight ability tests were used to assess the quality of mosquitoes over time to determine the maximum ferry duration that can be tolerated by the male mosquitoes before release. In addition, the recovery time for mosquitoes after chilling was investigated to determine the minimal release height.

## Genetics and molecular biology

### *Cytogenetic activities in support of sterile insect technique (SIT) applications and global trade*

For more than 30 years, cytogenetics has been an important component of IPCL's work, either with work performed 'in house' or through collaboration with external cytogenetics groups. Cytogenetics has supported SIT in different ways. The development, evaluation and improvement of Mediterranean fruit fly GSS has been facilitated by classical genetic linkage studies that helped to identify linked markers of importance, by the cytogenetic characterization of Y:A translocations, and by the isolation and characterization of inversions that increase GSS stability. Up to now, the quickest and most effective way to verify the identity of the medfly VIENNA 7 and VIENNA 8 GSS, as well as the presence of the D53 inversion, is polytene chromosome analysis. At the same time, and taking into account that a) chromosomal rearrangements are a common 'highway' to speciation in Diptera and b) when resolving relations among closely related species, multidisciplinary approaches are needed, cytogenetics has supported species delimitation in different Tephritidae species complexes. In collaboration with Profs. Antigone Zacharopoulou, Penelope Mavragani and Elena Drosopoulou from Greece, cytogenetic work has progressed in different fruit fly taxa. The complete polytene chromosome map and the mitotic karyotype of the first member of the *Ceratitis* FAR complex (*C. fasciventris* F2) have been published (Fig. 13, top left) (doi: 10.1038/s41598-017-05132-3) and

molecular, genetic and cytogenetic analysis of two additional members of the complex is ongoing (Fig. 13, bottom left, top right, bottom right), along with analysis of F1 hybrids among them. These data
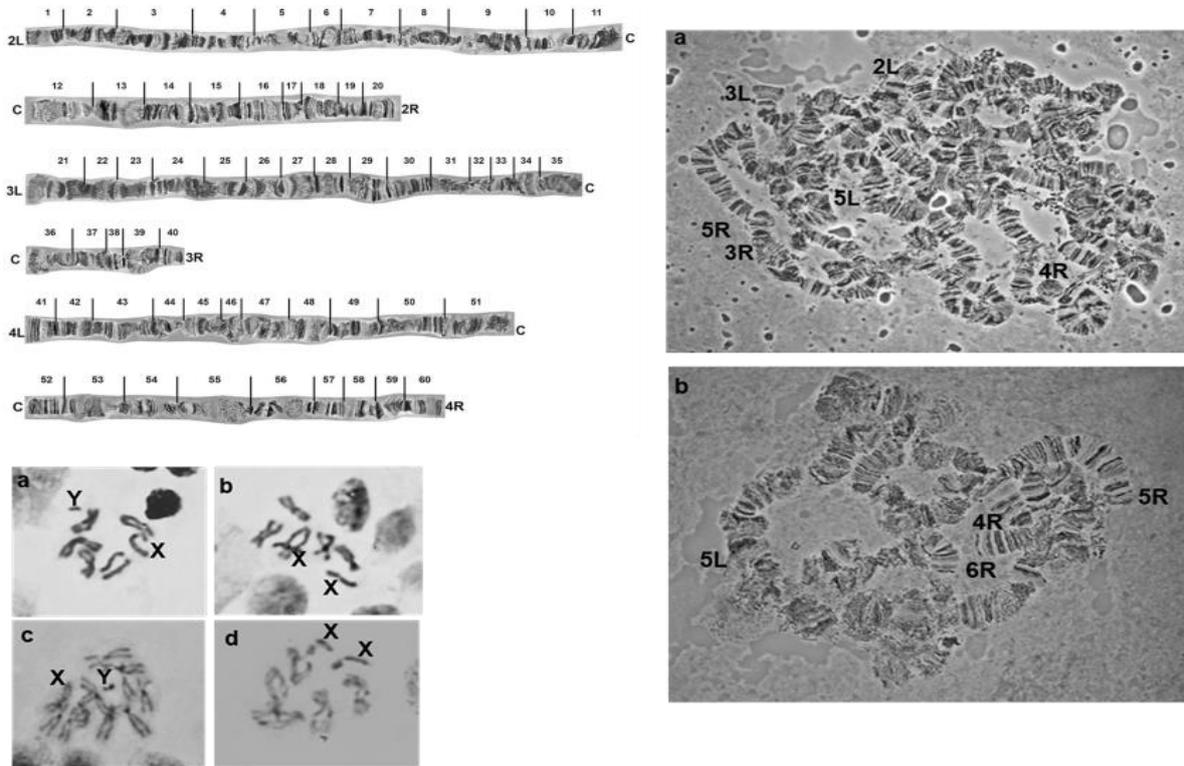


FIG. 13: (top left) polytene elements 2, 3, and 4 of the polytene chromosome map of C. fasciventris *F2, (bottom left) mitotic karyotypes of* C. rosa *(a, b) and* C. quilicii *(c, d): a, c: male; b, d: female, (top right) polytene nucleus of* Ceratitis quilici*. The chromosome arms 2L, 3L, 3R, 4R, 5L, and 5R, are indicated, (bottom right) polytene nucleus of* Ceratitis rosa*. The chromosome arms 4R, 5L, 5R and 6R are indicated*
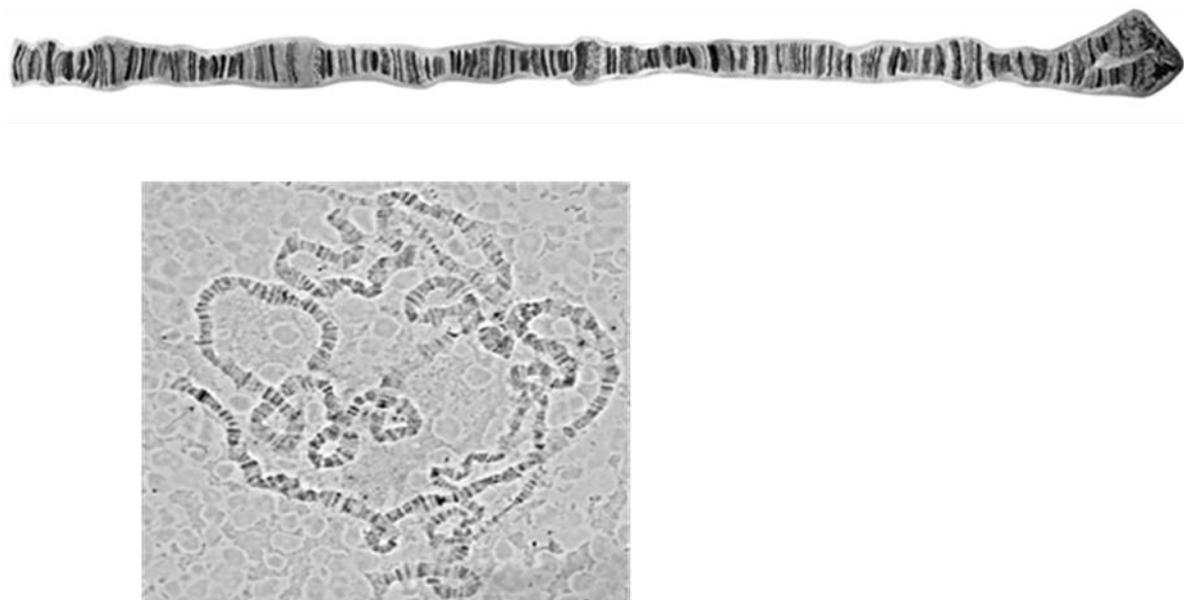


Fig. 14: (top) Polytene chromosome map of D. suzukii *under construction: the X chromosome, (bottom) In situ hybridization on* D. suzukii *polytene chromosomes to provide characteristic landmarks for all polytene elements. Clone pcr60A hybridization in region 60 of the 2R polytene arm. The arrow points to the hybridization signal*

are expected to contribute to the delimitation of this species complex, combined with work performed in other institutes in different research fields, under the frame of integrative taxonomy. Mitotic and polytene chromosome analysis is ongoing in two *Bactrocera* species (*B. correcta* and *B. zonata*) and one *Zeugodaucus* species (*Z. tau*). Finally, substantial progress has been made in the cytogenetic analysis of another major invasive agricultural pest species, *D. suzukii*. The polytene chromosome map of this species is under construction (Fig. 14, top), while preliminary *in situ* hybridization experiments on polytene chromosomes gave promising results (Fig. 14, bottom).

### *Towards the development of a SIT-based package against* Drosophila suzukii

*Drosophila suzukii* is a major invasive agricultural pest species, which has spread from west to east USA, Canada and most of the Mediterranean region. There are ongoing efforts in the IPCL to develop the SIT package against this major pest species. Interestingly, natural populations of *D. suzukii* are infected with the symbiotic bacterium *Wolbachia*, which is known to be naturally present in numerous insect species and to be associated with reproductive alterations such as cytoplasmic incompatibility, which is a conditional male sterility. In the frame of the SUZUKILL project, and in collaboration with colleagues at the BOKU University in Vienna (Prof. Christian Stauffer) and at the CNRS/LBBE in Lyon (Drs Laurence Mouton, Patricia Gilbert and Fabrice Vavre), we are investigating whether *Wolbachia* symbiosis and *Wolbachia*-induced cytoplasmic incompatibility (CI) could be combined with irradiation, potentially resulting in high-quality sterile males to be used in a combined SIT/IIT approach in a way similar to that developed for the suppression of *Aedes albopictus* and *Ae. aegypti* mosquito vector populations. During the last year, we have compared the life history traits of naturally *Wolbachia*-infected lines with *Wolbachia*-free lines of *D. suzukii*, which have been produced by antibiotic treatments. This initial comparative analysis has provided evidence that *Wolbachia* infection has a cost, a finding that needs to be taken into consideration in determining whether an infected or an uninfected line should be used for the mass-rearing and potential SIT applications against this major pest.

## CAPACITY BUILDING AND SERVICES

In 2017, the IPCL hosted 12 cost-free experts (CFE) and 12 consultants (C) (of which 5 were PhD students), six interns, ten fellows (F) and one scientific visitors (SV) (the latter two categories funded by the IAEA's Department of Technical Cooperation) in the following areas:

| Name | Country | Status | Duration | Topic |
|---|---|---|---|---|
| KOSKINOTI, Panagiota | Greece | Intern/CFE | 6 mth/ 1 mth | Mosquito genetics, and olive fruit fly molecular biology and symbiosis work |
| RIVERA, Camilo | Guatemala | Intern | 7 mth | Phytosanitary treatments |
| GUILLEN RIVERA, Martha | Mexico | Intern | 9 mth | Characterising GSS of fruit flies |
| PORRAS, Mitzy | Colombia | Intern | 5 mth | Characterising GSS of fruit flies |
| COUTINO MORENO, David | Mexico | Intern | 6 mth | Phytosanitary treatments |
| AMORIM DA SILVA CARDOSO, Amanda | Brazil | Intern | 1 mth | Phytosanitary treatments |

| Name | Country | Status | Duration | Topic |
|---|---|---|---|---|
| BUSTOS, Emilia | Mexico | C | 2 wks | Phytosanitary database |
| ANANTANAWAT, Juniwat | Australia | CFE | 1 mth | Fruit fly genetics |
| DEMIRBAS, Gueler | Turkey | C (PhD) | 12 mth | Tsetse symbionts and pathogens |
| MEKI, Irene | Kenya | C (PhD) | 12 mth | Management strategies for tsetse pathogens |
| SANOU, Guy | Burkina Faso | C | 6 mth | Rearing of tsetse |
| HARRISON, Sarah | Canada | CFE | 3 mth | Rearing of fruit flies |
| SASSU, Fabiana | Italy | CFE (PhD) | 12 mth | Radiation biology and rearing of *Drosophila suzukii* |
| NIKOLOULI, Katerina | Greece | CFE (PhD) | 12 mth | *Wolbachia* in *Drosophila suzukii* |
| WALLNER, Thomas | Austria | CFE | 6 mth | Rearing of mosquitoes |
| SALVADOR HERRANZ, Gustavo | Spain | CFE | 2 mth | Automation rearing techniques for mosquitoes and tsetse flies |
| CARAVANTES, Silvana | Guatemala | CFE | 5½ mth | Rearing fruit flies |
| CULBERT, Nicole | UK | C (PhD) | 12 mth | Mosquito handling techniques |
| DIAS DE CASTRO, Vanessa | Brazil | CFE | 6 mth | Phytosanitary treatments |
| DICKO, Ahmadou | Mali | CFE | 2 wks | Mosquito and tsetse modelling |
| TUR LAHIGUEIRA, Carlos | Spain | C | 2 wks | Development of drones for mosquitoes |
| AGUADO QUINONES, Angela | Spain | C | 2 wks | Development of drones for mosquitoes |
| ALMENAR GIL, David | Spain | C | 2 wks | Development of drones for mosquitoes |
| LYRAKIS, Emmanouil | Greece | CFE | 1 wk | Fruit fly genetics |
| WOODS, Bill | Australia | CFE | 2 wks | Mating behaviour of fruit flies |

| Name | Country | Status | Duration | Topic |
|---|---|---|---|---|
| SCHUTZE, Marc | Australia | CFE | 2 wks | Mating behaviour of fruit flies |
| CHEN, Shi | China | F | 1 mth | Mosquitoes |
| MONZON, Jorge Torres | Mexico | F | 7 mth | Mosquitoes |
| MASHATOLA, Thabo Rhine | South Africa | F | 12 mth | Mosquitoes |
| DIAS DE CASTRO, Vanessa | Brazil | F | 6 mth | Fruit flies |
| KHEDER, Noman | Sudan | F | 5 mth | Mosquitoes |
| ZAKIA, Al Alawi | Oman | F | 3 mth | Fruit flies |
| JEANNE DOR, Ariane Liliana | Mexico | F | 10 d | Mosquitoes |
| NAHALLAGE DONA, Asha | Sri Lanka | F | 6 mth | Mosquitoes |
| ZHARMUKHAMEDOVA, Galiya | Kazakhstan | F | 10 d | Fruit flies |
| WEERAWAN, Sukamnouyporn | Thailand | F | 4 mth | Mosquitoes |
| JIASNESH, Anish Ram | Fiji | SV | 10 d | Fruit flies |

In 2017, the GMB group maintained 13 species of fruit flies and 182 mutant strains or populations, and four species of mosquitoes with in total 38 strains/colonies/populations.

The PP group maintained 95 different fruit fly species/strains or populations, the LP group seven tsetse species and the HDV group four mosquito species and 12 mosquito strains.

The GMB/PP groups carried out 47 fruit fly shipments to 20 institutions in Austria, Bangladesh, France, Germany, Greece, Guatemala, Italy, Mexico, Panama, Senegal, Spain and Sweden and six shipments of preserved fruit flies to six institutions in Benin, Brazil, China, Italy, the Netherlands and the USA.

The LP group carried out 177 shipments of tsetse pupae (at total of 372 543 pupae of which 239 744 *G. palpalis gambiensis* to Senegal) to ten different institutions in Belgium, Burkina Faso, France, Greece, Senegal, South Africa, Uganda, UK and the USA.

The HDV group carried out 25 shipments of mosquitoes to nine institutions in French Polynesia, Germany, Italy, Spain, Sweden and Switzerland.

In 2017, the IPCL received 459 official visitors from 75 countries.

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# THE PLANT BREEDING AND GENETICS LABORATORY

# EXECUTIVE SUMMARY

Nuclear techniques are powerful tools to induce genetic variation in plants and enhance agrobiodiversity. They have enabled the development of superior mutant varieties with higher yields, tolerance to plant diseases and abiotic stresses such as drought, salinity and heat.

Food security and adaptation to climate change and variability continue to be the main drivers for the R&D activities at the Plant Breeding and Genetics Laboratory (PBGL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

In 2017, the PBGL conducted the following activities: (i) optimized phenotypic and molecular protocols for effective screening of mutants to contribute to climate-smart agriculture and food security; (ii) contributed to human and institutional capacity building of Member States in techniques used for the induction and screening of mutations, including efficiency-enhancing (bio)technologies; (iii) provided gamma and X-ray irradiation services to Member States where appropriate facilities are not available; and (iv) introduced the concept of a laboratory network to streamline capacity building efforts and catalyse R&D cooperation among Member States.

PBGL's R&D activities focused on developing mutation breeding tools and techniques for the control of the parasitic weed *Striga* and drought tolerance in cereals, improving feed quality in barley and combatting disease epidemics, including Panama disease in banana caused by *Fusarium oxysporum f. sp. cubense* Tropical Race 4 (Foc TR4) and the coffee leaf rust disease in coffee caused by *Hemileia vastatrix*. These activities were carried out in the context of ongoing Coordinated Research Projects (CRPs).

In the case of the *Striga* CRP D25005 on 'Mutation Breeding for Resistance to Striga Parasitic Weeds in Cereals for Food Security', laboratory- and greenhouse-based screening protocols for resistance to *Striga* in sorghum and upland rice were optimized. For the greenhouse protocol, various parameters (pot size, amount and depth of *Striga* seeds, watering schedule, and composition of soil) were optimized for reproducible infestation of the host plants by *Striga* in pot experiments. Under standardized conditions, differential sensitivity to *Striga* infestation between susceptible and tolerant controls could be reliably quantified. A laboratory method for *Striga* infection based on gels or rhizotron system was also developed. This method can be utilized for investigations of the *Striga* resistance mechanism in different mutant lines.

Under the CRP D23031 on 'Improving Resilience to Drought in Rice and Sorghum through Mutation Breeding' and CRP D23030 on 'Integrated Utilization of Cereal Mutant Varieties in Crop/Livestock Systems for Climate Smart Agriculture', we initiated two pilot projects to develop molecular markers for early maturing/semi-dwarf traits in sorghum and reduced lignin content in barley in 2016. These projects serve to establish the marker kits and protocols to enable marker-assisted selection/back crossing in mutation breeding programs of the Member States. In a next phase, similar tools and methods will be developed for additional priority crops and traits of economic importance to Member States.

A first project in this respect is focused on Wad Ahmed, a popular sorghum variety in Sudan, albeit that it matures slightly late and is (too) tall. Previously reported mutagenesis and breeding work have generated six semi-dwarf and early maturing advanced mutant lines. In 2017, $F_1$ crosses were performed between these six mutants and the parent line, and planted in the field. From these, two $F_2$ segregating populations were obtained and for each about 250 $F_2$ seeds were planted in pots in the greenhouse for further growth, phenotyping and seed set. Whole genome DNA sequencing of the parent and the six mutant lines was conducted and several dozen candidate sequence variants, including SNPs and INDELs between the mutant lines and parent, were identified with high confidence. As a next step, PBGL will undertake a genetic mapping experiment to identify the causal mutations.

Once known, these will be converted into molecular markers to facilitate introgression of these alleles into sorghum breeding programs in Member States.

Similarly, molecular markers are being developed for a feed quality trait in barley, *rob1*. Rob1 creates a reduced lignin content in barley, which improves digestion by animals. Previously, several deleterious mutations were identified in the *Cad2* gene as candidate causative mutations for the reduced lignin content. We have now developed a user-friendly, codominant genotyping assay for the Y28 mutant allele. Also, $F_1$ crosses were made and two $F_2$ populations are in the greenhouse for validation of the assay, phenotyping and linkage analyses. For both sorghum and barley, phenotypic analysis of the $F_1$ showed that the mutant traits are recessive. The availability of marker assays for recessive traits will greatly facilitate selection in breeding programs.

For banana, four candidate Foc TR4-resistant clones were obtained from the CRP counterpart of the Philippines and have been multiplied *in vitro* at the PBGL for genetic stability testing and TR4 resistance profiling. Further, a method for determining variations in ploidy levels using low coverage genome sequencing was adapted to gamma-irradiated banana. Using this method, the genome of Novaria, an early flowering mutant of Grande Naine that was released in 1995, was analysed. Grande Naine is a cultivar of the well-known commercially important Cavendish banana and has a triploid (AAA) genome. This study revealed the presence of Copy Number Variations (CNV) across the genome of Novaria, including a CNV on chromosome 5 reducing the ploidy level from three to two and spanning ≈3,8 Mbp comprising over 100 open reading frames.

The concept of a Laboratory Network for mutation breeding was introduced at a regional Technical Cooperation meeting RAF5076 on 'Improving Selected Seed Propagated Crops Using Mutation Breeding in Africa', the aim of such a network being to streamline capacity building efforts among Member States and to strengthen R&D cooperation in the development and application of plant mutation breeding for climate change adaptation. In the context of this meeting, participants were invited to share advanced mutant lines of important agronomic traits and to initiate collaborative genetic and marker-development studies to make these mutants more widely useful for Member States.

During 2017, the PBGL hosted three interns, 14 fellows and three scientific visitors covering in total 31 man-months of training on techniques for mutation induction, mutant population development and efficiency enhancing biotechnologies in support of climate-smart agriculture and food security.

We further hosted one regional training course on mutation breeding and efficiency enhancing technologies for 20 participants of IAEA technical cooperation projects (TCPs), mainly from Asia and Africa. In addition, the PBGL supported two national training courses in Oman and Qatar by providing technical support, protocols and irradiated seeds.

A total of 46 requests for plant irradiation from 27 Member States were handled in 2017. Of these, 21 requests were carried out in the context of CRP and TCPs.

## STAFF

| Name | Title |
| --- | --- |
| **Ingelbrecht**, Ivan | Laboratory Head |
| (vacant) | Plant Breeder/Geneticist |
| **Mukhtar Ali Ghanim**, Abdelbagi | Plant Breeder/Geneticist |
| **Matijevic**, Mirta | Laboratory Technician |
| **Jankowick-Cieslak**, Joanna Beata | Laboratory Technician |
| **Hofinger**, Bernhard[1] | Laboratory Technician |
| **Berthold**, Guenter | Field/Greenhouse Worker |
| **Ali**, Adel[2] | Laboratory Technician |
| **Goessnitzer**, Florian[3] | Laboratory Technician |
| **Warthmann**, Norman[4] | Consultant |
| **Mletzko**, Joanna Malgorzata | Team Assistant |
| **Jarc**, Luka[5] | Intern |
| **Akgun**, Eda[6] | Intern |
| **Shoukat**, Sadiya[7] | Intern |

[1] Separated in May 2017; [2] joined in June 2017; [3] joined in August 2017; [4] October to November 2017; [5] January to August 2017; [6] July to December 2017; [7] joined in August 2017.

# MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

## Mutation breeding for resistance to *Striga* parasitic weeds in cereals for food security

The parasitic weeds *Striga asiatica* and *Striga hermonthica* are major biological constraints to cereal production in most of sub-Saharan Africa and semi-arid tropical regions of Asia. *Striga* is particularly challenging because it infects the roots of its host plant and so remains invisible until the time when it emerges from the soil. By that time the damage to the host plant is already inflicted and thus conventional weed control measures cannot be applied.

The main objective of the CRP D25005 on 'Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security' is to develop laboratory, screenhouse and field screening protocols of mutant populations of sorghum and upland rice for resistance to *Striga asiatica* and *Striga hermonthica*. In addition, this CRP focuses on technologies such as the rapid generation cycling of crop plants, doubled haploid techniques and the use of molecular markers to enhance the efficiency of mutant identification and accelerate the delivery of resistant varieties.

In 2017, the PBGL developed a screening protocol for resistance to *Striga* parasitic weeds under glasshouse conditions by optimizing various parameters related to seedling growth and infestation by *Striga*. These parameters included pot size, watering regimes, temperature, preconditioning of the *Striga* seed as well as the concentration and distribution of the *Striga* seed in the soil. Parameters were optimized for sorghum and upland rice and conditions for the successful infestation of susceptible plants by *Striga* were optimized (Fig. 1).



FIG. 1: Screening in pots for resistance to Striga in sorghum (A, left pot infected) and upland rice (B, right pot infected) compared to healthy plants of the same variety

These protocols are now being validated by screening M$_2$ populations of sorghum made available by participants of this CRP.

In addition, laboratory methods for infestation of rice and sorghum by *Striga*, using rhizotron and gel-assay methods, were developed (Fig. 2). These assays will be utilized to study allelism and mechanisms of resistance to classify the different *Striga* resistance mechanism. Accelerating techniques, such as rapid cycling of crop generation and efficiency enhancing technologies using genomics and molecular
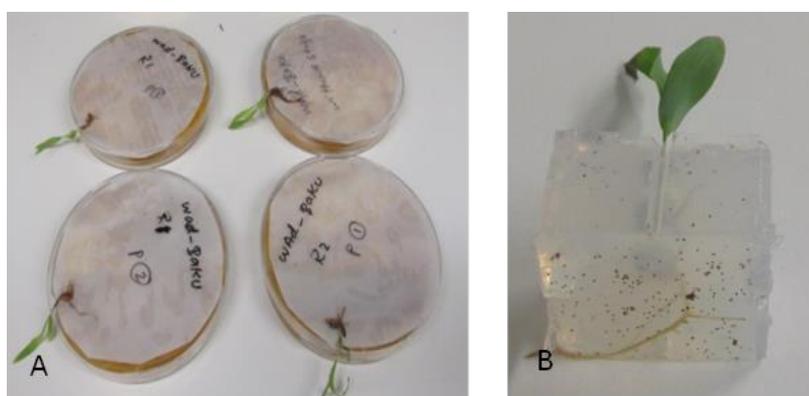


Fig. 2: Rhizotron (A) and gel-based assay (B) for Striga infestation

markers, will be adapted for rapid the delivery of durable resistance through gene pyramiding.

## Genetics and marker development for climate-smart agriculture and food security

In 2016 the PBGL initiated a programme to develop molecular markers for important mutant traits to facilitate their wider utilization by Member States. The initiative started with pilot examples in sorghum and barley for semi-dwarf/early maturing and improved feed quality, respectively. These two pilot cases serve to establish and validate the protocols and marker kits that will subsequently be shared with Member States before being gradually expanded to other priority crops and traits of importance to individual countries and regions.

### *Marker development for an early-maturing and semi-dwarf trait in sorghum*



*FIG. 3: View of the field trail of the six mutants and the Wad Ahmed at the PBGL field*

A semi-dwarf and early-maturing mutant trait in sorghum was chosen as one of two pilot projects for marker development at the PBGL. The mutation was induced by gamma irradiation in a tall farmer-preferred sorghum variety, Wad Ahmed, from Sudan. In total, six mutant sorghum lines, D1 through D6, were advanced to the M7 stage. The trait is thought to be recessive and to be controlled by one major gene with potential modifiers. The mutant is associated with early maturity and enhances stay-green at maturity, which is useful for tolerance to terminal drought and in forage sorghum production. The mutants are useful as an agronomically

important trait for semi-dwarf plant height that reduces yield loss caused by lodging, enhances response to fertilizer application, facilitates mechanized combine harvesting in large farming systems and is a critical trait in hybrid sorghum breeding. Based on the effects of similar mutations in other cereals, such as rice and wheat, the mutant is expected to improve yield and secure production in terminal drought prone areas. Since the semi-dwarf trait is considered recessive, development of a functional marker will facilitate rapid introgression of the mutant trait into farmer-preferred open-pollinated varieties and inbred lines for hybrid production.

The parental line was crossed with all six mutant lines and additional crosses were made between the mutant lines for allelism test. The $F_1$ generations were planted in the greenhouse and in the field in Seibersdorf, Austria along with replicated trials of the parent Wad Ahmed and the six advanced mutant lines (Fig. 3). The material was phenotyped for the mutant traits, i.e. plant height

**Sorghum mutant line D2**

(Field grown Oct 2017)

**Sorghum mutant line D3**

(Glass-house grown Oct 2017)
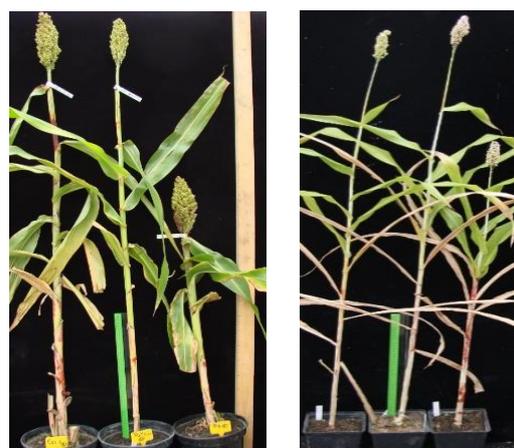


WT    F1    D2    WT    F1    D3

*FIG. 4: Sorghum parental line (WT) compared to mutant lines D2 and D3 with the $F_1$ hybrid (F1) showing the $F_1$ plant is tall as the parent*

and flowering time. Analysis of the $F_1$ generation showed that the semi-dwarf trait is recessive, as illustrated for mutant lines D2 and D3 (Fig 4).

We then conducted whole-genome re-sequencing of the parental line and the six mutant lines D1 to D6 to locate sequence variations between the parent and mutant lines using cost-effective 2nd generation DNA sequencing technologies and the ever-growing genomics resources that the global community of sorghum researchers makes publicly available. Several thousand candidate sequence variants, including SNPs, small INDELs and large coverage differences representative of large INDELs, were identified with high confidence (see Fig.5).
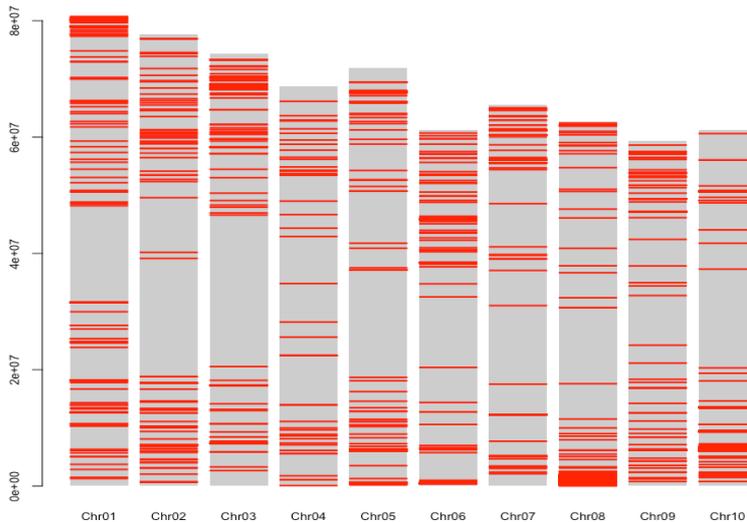


*FIG. 5: High quality sequence variants (red horizontal lines) distinguishing control and early maturing/semi-dwarf sorghum plants across the 10 chromosomes*

Because of the large number of sequence variants that are spread over the genome we will now proceed to initiate a genetic mapping experiment; by phenotyping and genotyping the segregating populations we can zero in on the region in the genome where the causal mutation resides. Once the causal mutations are known, these will be converted into molecular markers to facilitate introgression of these alleles into sorghum breeding programs by Member States using marker-assisted backcrossing. Furthermore, combining marker selection with PBGL's rapid cycling cultivation protocol in sorghum, allowing four cycles per year, introgression should be achieved in about two years, hence enabling significant gains in both time and cost.

This project links with the ongoing CRP D23030 on Integrated Utilization of Cereal Mutant Varieties in Crop/livestock Production Systems for Climate-smart Agriculture (see below) and the recently initiated CRP on D23031 on Improving Resilience to Drought in Rice and Sorghum through Mutation Breeding. The associated protocols will also be useful in the context of CRP D25005 on Mutation Breeding for Resistance to *Striga* Parasitic Weeds in Cereals for Food Security.

## Marker development for a reduced-lignin mutant trait in barley

The orange lemma mutation (*rob1*) in barley results in reduced lignin content and has applications for animal feed due to its higher digestibility. This project is carried out under CRP D23030 on Integrated Utilization of Cereal Mutant Varieties in Crop/livestock Production Systems for Climate-smart Agriculture in cooperation with CRP counterpart, Prof Grausgruber, BOKU, Austria. Previously, BOKU conducted *in vitro* feeding studies using the Hohenheim gas test and the rumen simulating technique (RUSITEC), which confirmed that the *rob1* mutant confers higher digestibility as feed for cattle compared to the wild type. The *rob1* mutation appeared for the first time in a barley accession from the Krasnodar region in Russia as a spontaneous mutation. Later, the orange lemma trait was induced by various mutagens in Swedish barley varieties. The trait is considered recessive and located on chromosome 6.

The PBGL identified the *cad2* gene encoding cinnamyl-alcohol dehydrogenase in the lignin biosynthesis pathway as the most likely candidate gene harbouring the causative mutations for reduced lignin. An additional gene (COMT) could also be involved. In total six different point mutations were identified in the *cad2* gene, all predicted to have deleterious effects on *cad2* gene expression.

The COMT gene was amplified and sequenced from different mutants; no mutations were detected, suggesting that this gene is not responsible for the *rob1* phenotype. $F_1$ crosses between wild type parental lines Optic and



*Fig. 6: Phenotype of lemma in the wild type parent (Optic) compared to mutant* rob1 *and two F1 hybrids*

Bowman and four different mutant lines were conducted. Five $F_1$ plants were obtained, three in a Bowman genetic background and two in an Optic background. Phenotyping of the $F_1$ plants confirmed that *rob1* is recessive (Fig. 6).

Further, an allele-specific marker assay was developed for the Y28 mutant allele of the *cad2* gene. The Y28 mutation creates a stop codon at amino acid position 28 of exon 1 of the *cad2* gene. The marker assay was adapted for an agarose gel system to enable easy analysis and interpretation. The genotyping assay is codominant and can reliably differentiate the wild type from mutant allele. Two $F_2$ populations are being grown in the greenhouse and will be used to validate the assay and for marker phenotype linkage analysis (Fig. 7).



*Fig 7: $F_2$ barley segregating population derived from a cross between wild type barley Bowman and the* rob1 *mutant PBGL1254*

## Combatting fungal diseases in coffee and banana

In case of coffee, seed stocks from four different Member States were used to optimize sterilization procedures. Further, radio-sensitivity experiments were initiated on embryogenic callus of two *C. Arabica* varieties using gamma rays in cooperation with the CRP counterpart Prof. Laimer, BOKU, Austria. These calli are currently being sub-cultured *in vitro* to evaluate their regeneration response.

Four putative Foc TR4-resistant banana clones have been obtained from Dole, Philippines and are being mass propagated *in vitro* at the PBGL. These materials will be used to verify the



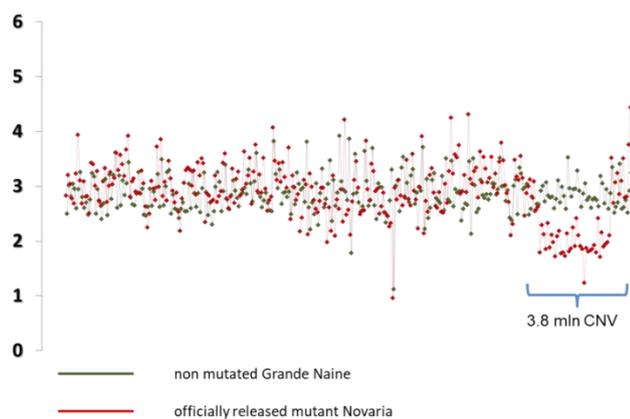*FIG. 8: Relative sequence read coverage (RSRC) plot of non-mutagenized Grande Naine (green) and Novaria (red) for detection of gamma-induced copy number variation (CNV) for chromosome 5. The RSRC values were set to 3 for the triploid control sample Grande Naine. The value 2, unique to mutant Novaria, indicates a ploidy drop from 3 to 2 in this region, corresponding to a putative deletion of 3.8 Mbp*

resistance phenotype and genetic stability testing in different hot spots in Member States to evaluate their potential use by Member States. In addition, the PBGL has developed a method based on low coverage whole genome sequencing to identify large genomic INDELs caused by gamma treatment in banana. This method was applied to Novaria, an early flowering gamma-ray induced mutant of Grande Naine. Grand Naine is a cultivar of the well-known Cavendish banana, commercialized as Chiquita banana. The genome analysis of Novaria revealed the presence of multiple copy number variations (CNV) that are spread across all chromosomes, including a deletion of approximately 3.8 Mbp on chromosome 5 spanning over 100 open reading frames (Fig. 8).

# CAPACITY BUILDING AND SERVICES

### Regional Meeting on 'Improving Selected Seed Propagated Crops Using Mutation Breeding in Africa', 3-5 May 2017, Vienna and Seibersdorf, Austria

A Regional Meeting on 'Improving Selected Seed Propagated Crops Using Mutation Breeding in Africa', was held on 3-5 May 2017 under RAF5076 'Improving Crops Using Mutation Induction and Biotechnology through a Farmer Participation Approach'. The meeting was attended by one representative each from ten African Member States. This meeting served to identify priorities for human capacity building at the PBGL of the Member States through Technical Cooperation Projects and, secondly also to launch the concept of a laboratory network for mutation breeding to facilitate R&D cooperation and streamline capacity building between the PBGL and these Member States. Initial focus is on the seed-propagated crops rice, sorghum and cowpea. The PBGL provided an overview of its R&D activities while the status of the mutation breeding program including mutant germplasm available under RAF5076 was jointly reviewed. The following three classes of mutant lines were identified: (i) early stage mutants corresponding to M1 and M2; (ii) middle stage corresponding to M3 and M4; and, (iii) advanced mutant lines (M5 and beyond) plus released mutant varieties. In case of Burkina Faso, Zambia, and Zimbabwe priority areas and modalities for capacity building with the PBGL were identified to be implemented in 2018-2019.

### Indonesia's Centre for Isotopes and Radiation Application becomes an IAEA Collaborating Centre

Indonesia's Centre for Isotopes & Radiation Application (CIRA/BATAN) officially became an IAEA Collaborating Centre in November 2017. The Collaborating Centre agreement will further strengthen collaboration between the PBGL and CIRA/BATAN on the development of plant mutation breeding and related nuclear technologies aimed at enhancing capabilities in crop improvement for climate change adaptation. Focal crops include rice, sorghum and soybean. In the context of these new linkages, a working collection of mutants and parental lines for verification is currently being established at the PBGL to support marker development for agronomically important traits.

### Regional training course on 'Induced Mutations and Supportive Biotechnologies for Cereal Breeding', 8-19 May 2017, Seibersdorf, Austria

This two-week regional training course was implemented under TCP RAS/5/74 on 'Supporting Mutation Induction and Supportive Breeding and Biotechnologies for Improved Wheat and Barley - Phase III' and was attended by 19 participants from 11 Member States (Burkina Faso, Iraq, Jordan, Lebanon, Lesotho, Oman, Qatar, Saudi Arabia, Syrian Arab Republic, Yemen and Zimbabwe). Lecturers, practical classes and demonstrations covered mutation induction and development of mutated populations; screening protocols for biotic and abiotic stresses (wheat rust, drought, heat stress and salinity) in cereals as well as efficiency enhancing technologies (rapid generation cycling, doubled haploid technologies, flow-cytometry and near-infrared spectroscopy [NIRS] for seed-quality analysis) and DNA sequencing, bioinformatics and molecular techniques for the development of molecular markers and marker-assisted selection in plant breeding. Several participants voiced an interest in joining a laboratory network on mutation breeding

## Fellowships and Scientific Visitors

During 2017, the PBGL hosted 12 fellows (F) and three scientific visitors (SV) who were trained in the following areas:

| Name | Country | Status | Duration | Topic |
|------|---------|--------|----------|-------|
| **Mheni**, N | Tanzania, United Rep. of | F | 2 months | Doubled haploid, *Striga* screening |
| **Hassan**, Omar | Sudan | F | 7 weeks | *Striga* screening protocol |
| **Kabore**, Nèbpawindé | Burkina Faso | F | 3 months | Mutation induction, marker-assisted selection |
| **Doubro Bombi**, Arnaud | Central African Republic | F | 3 months | Mutation induction, marker-assisted selection |
| **Sefume**, Lesole | Lesotho | F | 3 months | Mutation induction, marker-assisted selection |
| **Al Hinai**, Marwa | Oman | F | 3 months | Doubled haploid, marker-assisted selection |
| **Matova**, Prince | Zimbabwe | F | 2 weeks | Mutation induction, mutant population development |
| **Nunekpeku**, Wonder | Ghana | F | 6 months | Mutation induction, *in vitro* techniques |
| **Adam**, Mohamed | Sudan | F | 3 months | *Striga* screening |
| **Al-Mamari**, Alghaliya | Oman | F | 2 months | Marker-assisted selection |
| **Boureima**, Seyni | Niger | F | 1 month | *Striga* greenhouse screening protocol, EMS mutagenesis, marker-assisted selection |
| **Hamissou**, Zangui | Niger | F | 1 month | PCR and primer design, marker-assisted selection |
| **Bassole**, Ismael Henri Nestor | Burkina Faso | SV | 1 week | Mutation induction, mutant population development |
| **Elazazi**, Elsayed | Qatar | SV | 2 weeks | Mutation induction, mutant population development |

| Name | Country | Status | Duration | Topic |
|---|---|---|---|---|
| **Landau**, Alejandra | Argentina | SV | 1 week | Mutation discovery |

### *Irradiation services*

The PBGL received a total of 51 requests for plant irradiation from 29 Member States in 2017, covering 34 different plant species. Of these, 24 requests were received in the context of CRPs, TCPs or fellowships (F), with the remaining 27 requests from stakeholder institutions. In many cases, PBGL carried out associated radio-sensitivity test to determine the optimal irradiation dose for mutation induction. The total number of irradiation requests since 1977 now stands at 1540.

| Request no. | Country | Request type | Crop |
|---|---|---|---|
| 1495 | Germany | | Ornamental |
| 1496 | Burkina Faso | CRP | Sorghum, rice |
| 1497 | Germany | | Cassava |
| 1498 | Romania | TCP | Pea |
| 1499 | UK | | *Brassica napus* (rape) |
| 1500 | Sierra Leone | TCP | Rice |
| 1501 | Uzbekistan | | Paulownia |
| 1502 | Sudan | F | Pearl millet, sorghum, tomato, onion, cowpea |
| 1503 | Uzbekistan | | Cotton |
| 1504 | Tanzania | F | Maize |
| 1505 | Hungary | | Ornamental |
| 1506 | Poland | | Conifers |
| 1507 | Austria | | Chamomile |
| 1508 | Côte d'Ivoire | TCP | Maize |
| 1509 | Austria | | *Cannabis sativa* |
| 1510 | Iraq | TCP | Rice |
| 1511 | Iraq | TCP | Watermelon, squash, cucumber, okra, eggplant |

| Reque st no. | Country | Reque st type | Crop |
|---|---|---|---|
| 1512 | Senegal | TCP | Cowpea |
| 1513 | Namibia | TCP | Cassava, sorghum |
| 1514 | Germany | | Wheat |
| 1515 | the Netherlands | | Ornamental |
| 1516 | Germany | | Ornamental |
| 1517 | Lesotho | F | Beans |
| 1518 | Sudan | F | Sorghum |
| 1519 | the Netherlands | | Ornamental |
| 1520 | Oman | F | Garlic |
| 1521 | Ghana | F | Oil palm |
| 1522 | Bulgaria | TCP | Wheat |
| 1523 | Iraq | TCP | Cowpea |
| 1524 | Cameron | CRP | Maize, okra |
| 1525 | Germany | | Ornamental |
| 1526 | Romania | TCP | Barley |
| 1527 | the Netherlands | | Ornamental |
| 1528 | UK | | Hosta |
| 1529 | Austria | CRP | Coffee |
| 1530 | Germany | | Ornamental |
| 1531 | Qatar | TCP | Wheat, barley, *Stipa capensis*, *Cenchrus ciliaris* |
| 1532 | Mali | CRP | Rice, sorghum |
| 1533 | Zimbabwe | CRP | Maize |

| Reque st no. | Country | Reque st type | Crop |
|---|---|---|---|
| 1534 | UK | | *Brassica napus* |
| 1535 | the Netherlands | | Ornamental |
| 1536 | Qatar | TCP | *Prosopis cineraria, Haloxylon persicum* |
| 1537 | Palestine | TCP | Sesame |
| 1538 | Burundi | TCP | Pear |
| 1539 | Zimbabwe | | Cowpea |
| 1540 | Malawi | | Ground nut CG7 and Chitala, soybean Tikolore and Makwacha, maize E62 and E63 |

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# THE SOIL AND WATER MANAGEMENT & CROP NUTRITION LABORATORY

## EXECUTIVE SUMMARY

The Soil and Water Management & Crop Nutrition Laboratory (SWMCNL) is part of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. It assists Member States in the development and transfer of isotopic and nuclear technologies to improve the resilience of farmers' communities to climate change through climate-smart agriculture, including the protection of soil and water resources and optimization of soil, water and nutrient management practices. The SWMCNL also helps Member States to be better prepared in responding to nuclear emergencies affecting food and agriculture, as well in remediating the impact of these events on soil and agricultural water resources.

In 2017, the SWMCNL conducted a wide range of activities: (i) it developed robust and affordable isotope, nuclear and related conventional techniques for climate-smart agriculture; (ii) supported the improvement of nuclear emergency preparedness and response in food and agriculture; (iii) trained technical staff and scientists from Member States in the use of nuclear and related techniques to improve agricultural water management; (iv) conducted isotope analyses for research and development, and for IAEA projects where analytical facilities are not locally available; and (v) provided quality assurance services to Member States.

The research and development activities at the SWMCNL included the validation of the use of plutonium isotopes as a new tracer for soil erosion studies and novel applications of compound-specific stable isotopes to identify sediment pathways across arable land, with emphasis on small basins of less than 10 hectares. Mobile cosmic ray neutron sensor technology was tested at a wide range of altitudes between 100 and 2600 m a.s.l., showing its potential for advising on agricultural water management also in agroecosystems with low accessibility. This technology was also validated to assess soil moisture heterogeneity in the landscape. Nitrogen-15 and carbon-13 isotope analysis in greenhouse gases, soil organic carbon and nitrate in water was improved. Important progress was made in nuclear emergency preparedness and response in food and agriculture. The IT platform of the Decision Support System for Nuclear Emergencies Affecting Food and Agriculture (DSS4NAFA) is entering the last stages of development before its scheduled beta release in July 2018. These activities are all essential in supporting the implementation of the six Coordinated Research Projects (CRP) of the SWMCN Subprogramme, two of which are coordinated by the SWMCNL.

A second major component of the work of the SWMCNL is its significant contribution to training and capacity building in Member States. The SWMCNL hosted 21 fellows, 6 interns and 1 PhD student from 24 countries, for training on the use of isotopic and nuclear techniques to improve nitrogen and agricultural water management and soil conservation in support of climate-smart agriculture, as well as nuclear emergency response.

One publication, "Cosmic Ray Neutron Sensing: Estimation of Agricultural Crop Biomass Water Equivalent", was published by Springer. This provides methods for improving the accuracy of area-wide soil moisture measurements by cosmic-ray neutron sensors through the estimation of water equivalents. Information was further communicated to Member States through 38 book chapters, conference papers and publications in international peer-reviewed journals.

The SWMCNL analysed a total of 4745 and 250 samples for stable isotopes and fallout radionuclides, respectively. Most analyses were carried out in support of research and development activities at the SWMCNL focusing on the design of isotope and nuclear techniques to improve soil and water management practices. An additional analytical focus was on $^{13}C$-$CO_2$ and $^{15}N$-$N_2O$ measurements using laboratory based laser isotope analysers.

## STAFF

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| **Torres Astorga**, Romina[9] | International Centre for Theoretical Physics Fellow |

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# MAJOR ACHIEVEMENTS IN RESEARCH AND DEVELOPMENT

The Soil and Water Management and Crop Nutrition Laboratory assists in the development and transfer of robust and affordable nuclear techniques to Member States to provide support to farmer communities in climate-smart agriculture. The transfer of these techniques aims at the improvement of soil, water and nutrient management practices for increased land productivity with a high resilience to climate change.

The SWMCNL also helps strengthen preparedness and response to nuclear or radiological emergencies affecting food and agriculture. This includes research and development activities on how to collect, analyse, monitor and make available information through efficient data management and spatial and temporal mapping of radionuclide concentrations in soil, water, plants and food.

## Climate-smart agriculture

Climate change has become a significant threat to agriculture. More frequent weather extremes, from drought to flooding, from frost to heat, affect food security, especially for the most vulnerable communities. These increasingly amplify land degradation and harvest failure. Agriculture can be affected by climate change, furthermore climate change can be further accelerated by greenhouse gas emissions from agricultural practices. Climate-smart agriculture is therefore needed to sustain food production under these challenging conditions. As a result, Member States are requesting more technical assistance and training in developing soil and water management practices for climate-smart agriculture.

### State-of-the-art in fallout radionuclides: Are $^{239+240}$Pu isotopes suitable tracers for soil erosion assessment?

Fallout radionuclides (FRNs) have long been used as soil redistribution tracers in assessing soil erosion. Over the last decades, the main FRNs used were (a) anthropogenic caesium-137 ($^{137}$Cs), (b) naturally occurring lead-210 ($^{210}$Pb$_{ex}$) and (c) naturally occurring cosmogenic beryllium-7 ($^{7}$Be). $^{137}$Cs is by far the most commonly used and mature technique for assessing mid- and long-term soil redistribution, because $^{210}$Pb$_{ex}$ requires the application of self-absorption corrections for its determination using gamma-ray spectrometry and it suffers from high uncertainty of measurement results at low activity concentrations. Likewise, because of its short half-life of two months, $^{7}$Be is unsuitable for long-term measurements.

Relatively few attempts have been made to exploit the full potential of plutonium (Pu) isotopes as soil redistribution tracers, especially when compared to the much more common use of $^{137}$Cs. One major obstacle were the analytical limitations related to the measurement of plutonium at environmental level. Until recent years, the traditional radiometric alpha-particle spectrometry technique and to some extent to the accelerator mass spectroscopy technique (AMS) were used. However, the advances in mass spectrometric techniques, with the development and improvement of the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) techniques, and the higher availability of those techniques in laboratories worldwide, opened the possibilities of using Pu isotopes for a wide range of applications, including the tracing of soil redistribution and sediment transfer. An additional obstacle in the use of plutonium as soil redistribution tracer in the past was the non-availability or the inadequacy of existing conversion models to convert $^{239+240}$Pu inventories to soil redistribution rates. This was rectified in 2016, with the development of new conversion model called MOdelling Deposition and Erosion rates with RadioNuclides (MODERN) was developed.

Even though only few studies exist, which use $^{239+240}$Pu as a soil redistribution tracer, this FRN seems to have major advantages and thus a great potential as "*the*" future tracer for soil redistribution assessment (see Table 1). Studies have shown that $^{239+240}$Pu has the advantage of having relatively low variability in reference site inventories, as it is generally not influenced by fallout originating from nuclear power plant (NPP) incidents. Furthermore, Pu has the advantage that the sensitivity of the

two main isotopes, i.e. $^{239}$Pu and $^{240}$Pu, because of their long half-lives (Table 1) remains essentially at the same level as when they were deposited. This is a major advantage in terms of its use as a reliable tracer of soil redistribution in future studies compared to $^{137}$Cs. $^{239+240}$Pu is also a more suitable tracer compared to $^{210}$Pb$_{ex}$, because of the low concentrations and the high uncertainty in $^{210}$Pb$_{ex}$ determination. Regarding analytical requirements and feasibility of approaches, $^{239+240}$Pu has the analytical advantage of higher accuracy as compared to $^{210}$Pb$_{ex}$ and higher sample throughput, in particular if ICP-MS techniques are used for its measurements.

*Table 1. Comparison of $^{137}$Cs and $^{239+240}$Pu as anthropogenic soil radiotracers (adapted from Alewell et al., 2017).*

| | $^{137}$Cs | $^{239+240}$Pu |
|---|---|---|
| Elemental characteristic | Alkali metal | Actinide metal |
| Origin(s) | Anthropogenic (nuclear weapon test fallout and nuclear power plant accident release) | Anthropogenic (mainly linked to nuclear weapon tests fallout and potentially nuclear power plant accident release affecting the areas next to the accident site) |
| Radioactive half-life | 30 years | 24 110 and 6561 years for $^{239}$Pu and $^{240}$Pu, respectively |
| Radiation emission | Gamma emitter (662 keV) | Alphas emitters; alpha energies of $^{239}$Pu and $^{240}$Pu (5157 and 5168 MeV, respectively) |
| Laboratory determination | Gamma-ray spectrometry | Alpha-particle spectrometry; mass spectrometry, mainly ICP-MS and AMS |
| Mobility in soil | Limited and reduced in fertile soil, strong binding to fine soil particles and organic matter | Slightly higher than other existing FRNs |
| Uptake by plants | Soil-plant transfer reduced in fertile soils | Negligible plant uptake as compared to all other FRNs |
| Currently investigated areas with this tracer | Worldwide application during 4 decades | Tested and validated since less than 1 decade in only a few countries |
| Scales of investigation | Plot to region | Field |
| Fallout occurrence and soil deposit | Worldwide, with higher deposit in northern hemisphere | Worldwide, with higher deposit in northern hemisphere |

Hence, $^{239+240}$Pu has all the potential to become the next generation soil redistribution tracer because of its long half-life, ensuring its long-term availability in the environment, its analytical advantage in terms of measurement precision and measurement time, and the relatively homogeneous distribution at reference sites.

A more detailed review and comparison of the advantages and limitations of Pu isotopes versus more mature FRN technique (e.g. $^{137}$Cs) has been published by Alewell *et al.* 2017 and was performed under CRP D1.50.17 on "Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agroecosystems".

### *Use of Pu isotopes as soil redistribution tracers in Austria: Test and validation at the Grabenegg reference site*

The first part of the SWMCNL's evaluation of Pu isotopes as soil tracers was carried out in Grabenegg, at the experimental research station of the Austrian Agency for Health and Food Safety. The yearly precipitation of the study area is approximately 690 mm and the site, characterised by gentle to steep slopes, is composed of Cambisol soil. Upon selection of a suitable reference site (i.e. ~100 m$^2$ undisturbed flat pasture), one soil profile was prepared for precise incremental radioisotope determination (3 cm increments to a depth of 24 cm) and 12 bulk soil cores (0-30 cm) were collected. The soil of this reference site has been characterised as silt loam with an average content of 19% sand, 62% silt and 19% clay. All soil samples were analysed for $^{137}$Cs content using gamma spectrometry at the SWMCNL and for $^{239+240}$Pu content using alpha spectrometry at the Centre National de l'Energie, des Sciences et Techniques Nucléaires (CNESTEN) in Morocco.

Preliminary results showed that in terms of areal activity (i.e. Bq m$^{-2}$), 79% of the $^{137}$Cs and 73% of the $^{239+240}$Pu are concentrated in the top 12 cm of the soil profile. As expected in a suitable reference site, the vertical distributions for both isotopes highlight an exponential decrease of their content with depth (Fig. 1). The initial $^{137}$Cs and $^{239+240}$Pu fallout in 12 core samples collected at the reference site
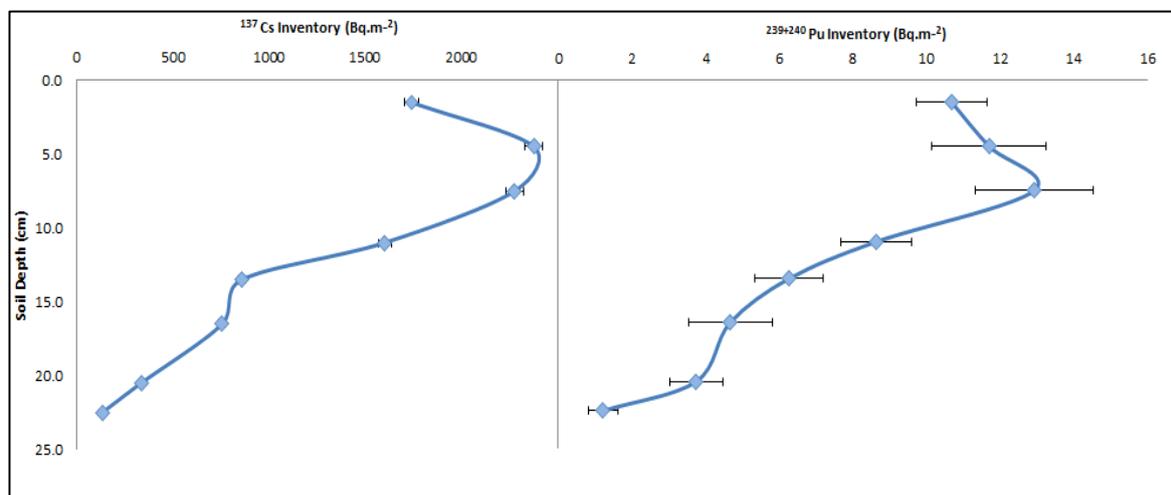


FIG. 1: Depth distribution for $^{137}$Cs (left) and for $^{239+240}$Pu (right) at the reference site. For both radioisotope profiles, the error bars represent the measurement uncertainty at 2 sigma

was evaluated to be 8179 ± 1794 Bq m$^{-2}$ (mean ± SD) with a coefficient of variation (CV) of 21.9% and at 56.1 ± 15.8 Bq m$^{-2}$ with a CV of 28.1%, respectively. Under the experimental conditions, the $^{137}$Cs and $^{239+240}$Pu baseline inventories were established with allowable errors (AEs) of 12.0% and 14.6% at 90% confidence level, respectively.

The first study related to the reference site confirms the possibility to use $^{239+240}$Pu as soil tracer due to its similar behaviour to $^{137}$Cs (specific vertical distribution and reduced spatial variability). An agricultural site under crop rotation was then identified at 600 m distance from the reference site,

which will be investigated in 2018 for multi-radioisotope determination and for assessing soil redistribution rates along its main slope direction.

This research is being performed as one of the contributions of the SWMCNL to CRP D1.50.17 on "Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agroecosystems".

### *Novel application of Compound Specific Stable Isotope (CSSI) techniques to establish on-site sediment origins across arable fields in Mistelbach, Austria*

The SWMCNL finalized the evaluation of the suitability and effectiveness of CSSI to track terrestrial sediment movement across arable fields through a case study at the small Austrian basin of Mistelbach (8.7 ha) located in Austria. In this study site, which is dominated by $C_3$ plants in limited rotation with $C_4$ species, a sampling approach involving composite sampling was used to identify potential sediment source materials from the four main agricultural fields. After selecting the best discriminant fatty acids (FAs), i.e. C22:0 = behenic acid; C24:0 = lignoceric acid, using one-way analysis of variance, correlation analysis and polygon analysis, mixing models were used to deconstruct the sediment mixture based on the isotopic signature results of the soil sources. Values of $\delta^{13}C$ signatures of both selected long-chain FAs (C22:0 and C24:0) and the bulk $\delta^{13}C$ of the source soils and sediment mixture were analysed with IsoSource and three Bayesian mixing models (i.e. Stable Isotope Analysis in R [SIAR], Mixing Stable Isotope analysis in R [MixSIAR] and Stable Isotope Mixing Models in R [SIMMR]). For all models, corrections based on the %$C_{org}$ of each source were used to convert the resulting isotopic proportions into soil proportions.

Depending on the stable isotopic mixing models used, our soil proportion results suggest that the source 3 and 4 (i.e. the main grassed waterway of the studied basin, also called 'thalweg') contributed the most to the sediment mixture in the deposition zone with 27.2 to 27.6 and 53.9 to 55.1%, respectively (Table 2). Results also indicate that the simpler IsoSource model performed as well as the other models for the sediment source apportioning and provided results like those from the more complex Bayesian stable isotope mixing models (see Table 2). The potential long residence time of the sediment in the thalweg (dominated by $C_3$ grass since its insertion in 2003), its "channelling" role as and its proximity to the sedimentation area can explain its major contribution to sediment deposition at the outlet of the basin.

*Table 2. Carbon soil proportion for each source to the mixture.*

| | IsoSource | SIAR | | MixSIAR | | SIMMR | |
|---|---|---|---|---|---|---|---|
| | Soil proportion (%) | Soil proportion (%) | SD (%) | Soil proportion (%) | SD (%) | Soil proportion (%) | SD (%) |
| Source 1 | 10.6 | 12.1 | 4.6 | 12.1 | 4.6 | 11.7 | 3.8 |
| Source 2 | 6.8 | 6.4 | 4.0 | 6.4 | 4.0 | 7.1 | 3.3 |
| Source 3 | 27.5 | 27.6 | 2.3 | 27.6 | 2.3 | 27.2 | 2.4 |
| Source 4 | 55.1 | 53.9 | 2.7 | 53.9 | 2.7 | 54.0 | 2.7 |

(*NB: SIAR was run using 50 000 iterations, MixSIAR using long run option and SIMMR with 5000 iterations*)

The results of our study highlight that over 80% of the sediment in the deposition zone at the basin outlet originates from the sources 3 and the source 4 (the thalweg). These two sources are the closest

sources to the mixture in the deposition zone and the results obtained are consistent with the current and past land uses. Both sources are the only ones with significant cultivation of maize in the last 22 years. As one of the most erosive crops, maize fields are prone to soil loss, especially at the beginning of the growing season when the soil is bare or considerably exposed to water erosion.

A previous investigation performed in 2008-2009 based on erosion plot records and ^{137}Cs determinations allowed us to assess soil redistribution magnitudes affecting the Mistelbach basin and to report a local maximum sedimentation rate reaching 50 t ha$^{-1}$ yr$^{-1}$. The conclusions of our recent study at the same experimental site emphasize that CSSI and FRN techniques are complementary for establishing land sediment redistribution. Their combined use could provide key decision support knowledge for optimized decision-making of land managers to ensure the sustainability of agroecosystem management.

Despite key advantages, applied CSSI studies using $\delta^{13}$C FAs are still limited. We therefore encourage the scientific community to test and validate this technique under additional agro-environmental situations and under different spatial scales.

This study, which involves three years of various inputs by the SWMCNL, was published by Mabit et al. 2017 as part of CRP D1.50.17 on "Nuclear techniques for a better understanding of the impact of climate change on soil erosion in upland agroecosystems".

## *Evaluating the effectiveness of mulch application to store carbon in agricultural soils: A follow-up study on short-term effects of mulch application on soil and microbial C and N in agricultural soils with different cropping systems*

Soils contain more carbon than plants and the atmosphere combined and increasing soil carbon content can provide multiple benefits, including climate change mitigation, improved soil quality and larger crop production. Depending on the type of farming techniques applied, agricultural soils can either store more carbon belowground, or further release carbon, in the form of $CO_2$, into the atmosphere. Mulch application is a farming practice that is frequently proposed to increase carbon content belowground and improve soil quality and it can be used in efforts to reduce greenhouse gas levels, such as in the "4 per 1000" Initiative launched by France on 1 December 2015 at the COP 21.

As a follow-up to our 2016 study, in which we tested the potential of mulch application to store carbon in agricultural soils with low and high organic carbon content (disturbed top soil from local Cambisols and Chernozems, respectively) in our SWMCNL greenhouse mesocosms, we also tested the effectiveness of mulch application to store carbon in the field (Fig. 2) with different cereal or legume-based cropping systems. This test was carried out at the SWMCNL long-term trial located in Grabenegg, Austria, which started in 2012.

A legume-maize rotation as well as sole maize monocrop or legume monocrop was maintained in the agricultural field experiment and plant residues from these productions were reapplied (at 1.0 t C ha$^{-1}$) or not to soils after harvest as mulch to mimic the practices of the farmer. After 5 years of maintaining this cropping system in the field, we sampled soil to measure carbon as well as determine the nitrogen pools and isotopic composition in the top 15 cm. It is postulated that soils with mulch would have higher soil carbon as well as nitrogen content and mulch application



*FIG. 2: Maize and vetch were grown at the SWMCNL long-term experiment in Grabenegg (Austria), which started in 2012. The site is characterised by Cambisol soils with low organic carbon content*

would have a greater effect on soils with legume-maize rotation due to the larger production of organic material by maize as well as legumes. We also postulated that $\delta^{13}C$ of soil organic carbon would be like that of the plants produced in monocrops but that the $\delta^{13}C$ would be more like the type of plant that contributed more carbon to soils in the legume-maize rotation and that this would likely to be the legume, since it has a lower carbon to nitrogen ratio that may have a more stabilizing effect for soil organic carbon.

Contrary to our postulation, soil carbon and nitrogen content did not improve with mulch application in the legume-maize rotation. This result was consistent with observations from the greenhouse mesocosms experiment. Interestingly, soil carbon and nitrogen content only improved in the field experiment with mulch application in the topsoil of maize monocrops (Fig. 3). $\delta^{13}C$ patterns of soil organic carbon further suggested that maize contributed more carbon to soils than the legume. The combination of the mesocosm greenhouse and field studies suggest that mulch added to maize monocrops, in the agroecological context of north-east Austria, can increase soil organic carbon but that the inclusion of legume production and legume mulch in crop rotation may reduce these benefits. Possibly, this effect is due to better decomposability of maize because of higher nitrogen availability from the legume crop in the rotation. However, this needs further testing.
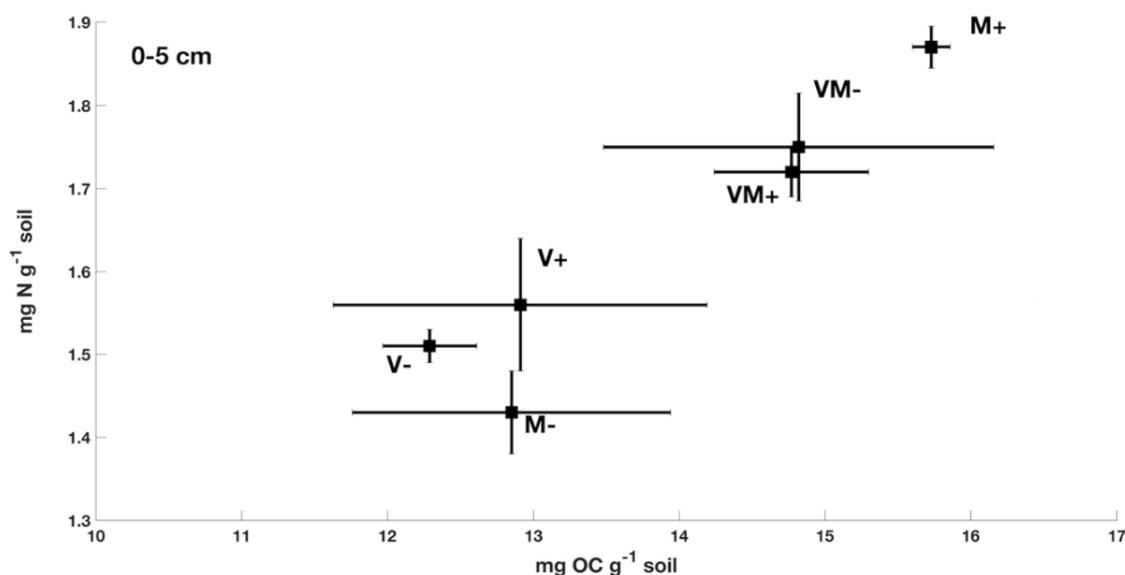


*FIG. 3: Soil organic carbon and total soil nitrogen content for 0-5 cm soils with sole vetch legume monocrops (V), sole maize monocrops (M) or vetch-maize rotation (VM) with (+) and without (-) mulch. Standard error bars for each cropping system with or without mulch are shown.*

This research was conducted under CRP D1.50.16 on "Minimizing Farming Impacts on Climate Change by Enhancing Carbon and Nitrogen Capture and Storage in Agro-Ecosystems".

## *Using laser spectroscopy to evaluate soil carbon loss and soil revival techniques*

Using SWMCNL's newly produced standard gases and laser spectroscopy techniques for $CO_2$ measurements, the SWMCN research team evaluated if we can revive stored soils and accurately measure soil carbon loss and its dynamics. If stored soils can be adequately revived, then soil carbon loss by soil respiration can be measured from typically selected fresh soils as well as archived samples that might be submitted to our laboratory by counterparts in Member States. To evaluate our soil revival technique, respiration rates of stored, revived stored and fresh soil types are being compared after mulch application in an incubation study. In addition to evaluating the soil revival technique, this study also estimates carbon loss using our homogenous $^{13}C$- labelled mulch compared with a pulse $^{13}C$-labelled mulch, both produced by the SWMCNL. To test this, non-labelled mulch, pulse labelled

mulch and homogenous labelled mulch was applied to each soil type in a factorial design and compared the differences in the $\delta^{13}C$ signal in respired soil $CO_2$ (Fig. 4).

Ultimately, these ongoing soil incubation studies will provide information on whether stored soils can be revived to gain information on rates of soil carbon loss without having to take measurements in the field or on freshly obtained samples. This will allow analysis to be performed on many soils that might normally be considered too distant from our FAO/IAEA laboratory. Additionally, the mulch application studies will provide more information on the rates of mulch-derived carbon
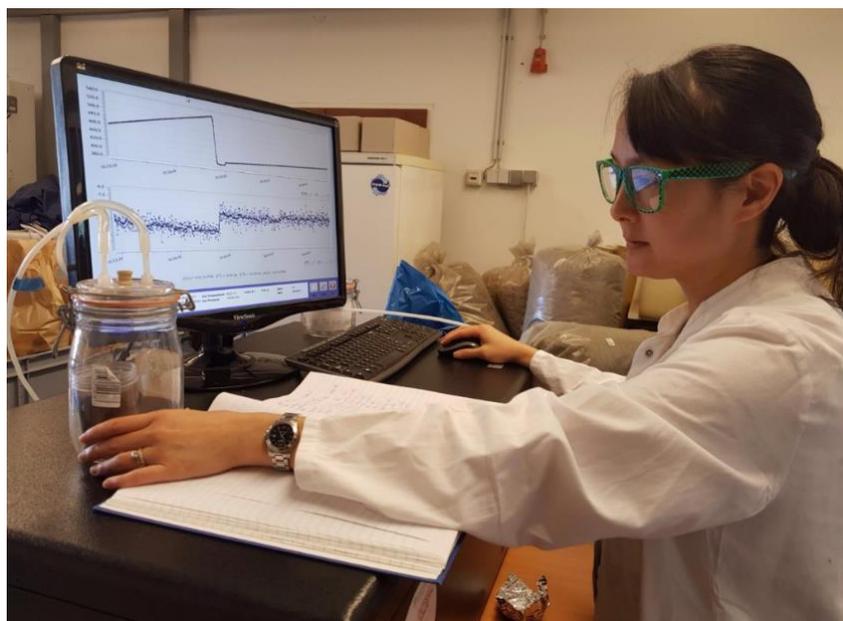


FIG. 4: Soil respiration rates and carbon-13 signature of respired $CO_2$ were measured using our established laser spectroscopy technique.

loss that are estimated using different types of carbon-13 labelled mulch.

This research was conducted under CRP D1.50.16 on "Minimizing Farming Impacts on Climate Change by Enhancing Carbon and Nitrogen Capture and Storage in Agro-Ecosystems".

## *Using stable isotopes to evaluate the influence of water stress on the water use efficiency and starter fertilizer utilisation rate of soybean*

The high nutritional and economic value of soybeans leads to a production increase and shifts to even arid and semi-arid climate zones. However, limited water supply and prolonged periods of water stress can have severe effects on crop growth and production. The goal for researchers and agronomists is to be able to select and breed more drought tolerant soybean varieties to better understand the implications of drought stress on crop performance. While breeding programs are often very time consuming, agronomic practices that focus on the efficient use of water resources can give almost immediate results. The carbon isotope discrimination (CID) technique and the nitrogen isotope dilution method were used to compare two different water regimes applied on soybeans.



FIG. 5: Daily gravimetric analysis. The pot surface is covered with blue plastic beads to minimize soil evaporation.

For this purpose, a pot experiment was conducted in a climate chamber, using the soybean variety Sigalia and summer wheat (*Triticum aestivum*) as a reference crop (Fig. 5). To ensure plant growth, all 12 replicates of each plant type were well watered and then divided into two sets: well-watered and limited watered. Stress symptoms became visible after 29 days after planting. The CID results showed $\delta^{13}$C values in the range of -27 to -30‰ for well-watered and -25 to -28‰ for stressed soybeans, indicating a clear and significant effect of water treatments on the isotopic signature. Water treatments showed a significant influence on the starter N utilisation rate with average values of 76% for well-watered and 27% for water-stressed soybeans (p<0.0001). At an average of 2.2 kg/m³, Water Use Efficiency (WUE) was highest for well-watered plants compared to 1.1 kg/m³ for stressed plants. Typically, stressed plants show an increase in WUE, but in this case a low yield with low transpiration resulted in a reverse situation, which asks for prudence in the extrapolation of our findings to real field conditions.

### *Area-wide soil moisture sensing at low and high elevation using the mobile cosmic ray neutron sensor technique*

The use of the Cosmic Ray Neutron Sensor (CRNS) for monitoring area-wide soil moisture (footprint of about 20 ha) has been the subject of multiple studies in recent years. The CRNS technology now exists in both a stationary and a mobile "backpack" form. The use of the mobile CRNS opens possibility for application in many diverse environments, in particular in situations with difficult accessibility, such as mountainous terrains. However, one major parameter that can influence the performance of CRNS is its altitude. With altitude, the air pressure changes and this affects the neutron counting.

In 2017, the SWMCNL team used a mobile 'backpack' CRNS device and measured its counts at both low and high elevations. Eight different altitudes from 100 to 2600 m a.s.l. were assessed on the effect of the atmosphere on cosmic-ray neutron raw counts. From the data collected it is clearly noted that neutron counts increase with elevation, implying higher footprint at higher attitude (Fig. 6), which agrees with published data[1]. Besides, the stability of the raw neutron counts is much better at high elevation compared to low elevation (low coefficient of variation; Fig. 7). In addition, the coefficient of variation and altitude show strong correlation ($R^2 = 0.98$). This relationship will be further validated in 2018.
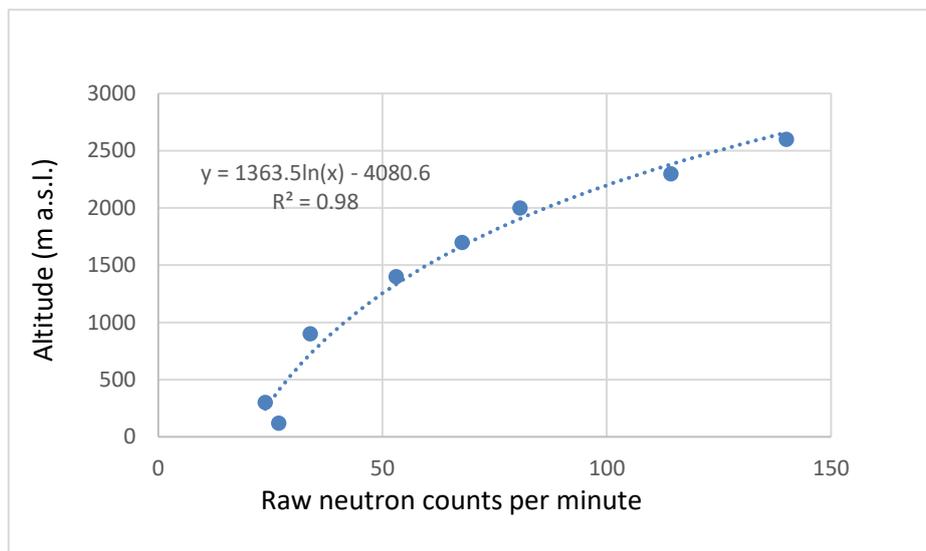


*FIG. 6: Raw neutron counts per minute of the mobile cosmic ray neutron sensor (CRNS) backpack at different elevations (from 100 to 2600 m a.s.l.) across study sites in Austria*

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[1] X Zhu, R Cao, M Shao, Y Liang (2018). Footprint radius of a cosmic-ray neutron probe for measuring soil-water content and its spatiotemporal variability in an alpine meadow ecosystem. Journal of Hydrology, 558: 1-8.
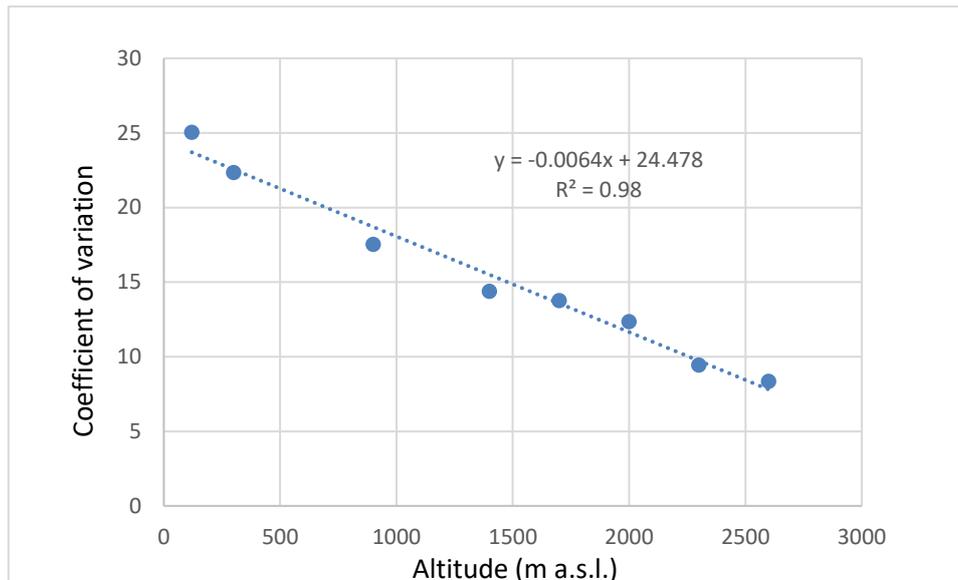
*FIG. 7: Coefficient of variation of the raw neutron counts of the mobile cosmic ray neutron sensor (CRNS) backpack at different elevations (from 100 to 2600 m a.s.l.) across study sites in Austria.*

During the 2017 field campaign, studies were conducted in the Rauris valley of the Austrian Alps at three different elevations within the Rauris watershed (900, 1400 and 1700 m a.s.l.). Calibrations of the 'backpack' CRNS were performed at each site along with data validation via volumetric soil moisture determination. Validation data from 2016 showed that the relationship between soil moisture data determined via *in-situ* Time Domain Reflectometry (TDR), volumetric soil moisture determination and that determined via the mobile CRNS is good (RMSE ~<2.5% volumetric). This was also confirmed in the 2017 dataset (Fig. 8), even on stony soils in the Rauris valley, represented by the red open circles. This demonstrates the suitability of the m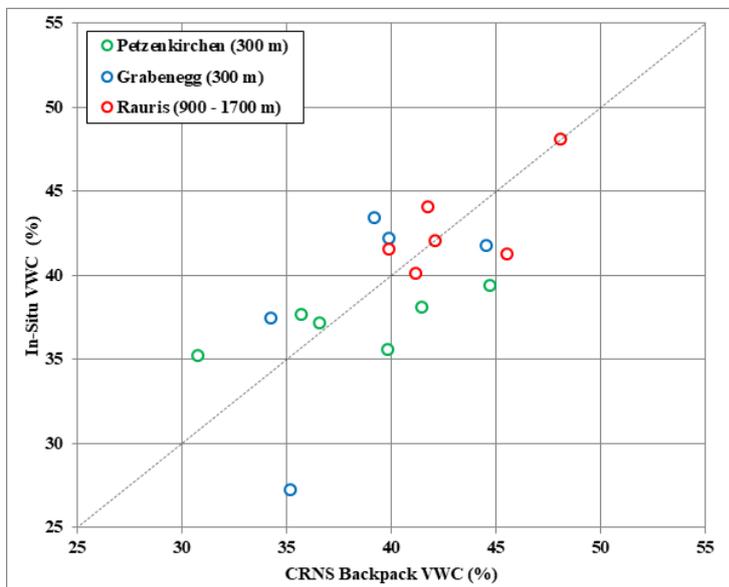obile CRNS under difficult landscapes not accessible or impossible using other soil moisture sensors including the stationary CRNS or traditional soil moisture point-sensor technology.

The efficacy of this technique in remote alpine landscapes shows potential for watershed hydrology and high elevation agricultural water management. However, further research is being carried out to fine-tune the use of this technique for these fragile agroecosystems.

This research was performed under CRPs D1.20.13 on "Landscape Salinity and Water Management for Improving Agricultural Productivity" and D1.50.17 on "Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems".



*FIG. 8: Comparison of* in-situ *soil moisture values (determined from traditional gravimetric soil sampling) and cosmic ray neutron sensor (CRNS) soil moisture values.*

### Soil moisture heterogeneity mapping by using mobile cosmic-ray neutron sensor technology

Over the last 5 years, new applications have been developed for rover-mobile cosmic ray neutron sensor (CRNS) technology to assess *in-situ* soil moisture heterogeneity. In the summer of 2017, a survey was performed at Grabenegg, Austria, to assess soil moisture heterogeneity using the backpack CRNS. The site is characterized by a hilly undulating landscape with moderate to severe slopes. The survey consisted of two linear transects of six measurement positions in a maize field and five positions in grassland. Fig. 9 shows the location of each of the 11 backpack points. At each location the backpack was set up on the ground and left running for a minimum of 15 minutes. For the first six locations a handheld 15 cm TDR sensor was used to measure soil moisture at ten spatial locations around the backpack CRNS. The latter has been shown to measure up to 30 cm depth. As such we would expect similar spatial trends but a different absolute soil moisture value. We also note that the soils in the grassland area were too hard to insert the rods without damaging them thus precluding us from sampling all 11 sites as intended to. This underscores a key advantage of the CRNS backpack's non-invasive observations and rapid use in all study sites.



FIG. 9: Location of 11 backpack survey points collected on 6th of July 2017 in Grabenegg Austria. The backpack was left at each location for ~15 minutes. A mobile TDR sensor (0-15 cm) was used at ten locations around each backpack survey point. The TDR system was only used on the first six survey points due to challenging soil conditions

Using the average $N_0$ value from the calibration procedure we could group the neutron counts by location and estimate soil moisture. Fig. 10 illustrates the estimate of CRNS soil moisture and its uncertainty due to counting statistics. Fig. 10 also shows the average TDR and uncertainty from ten repeated samples around the backpack. As expected the soil moisture values follow the same general pattern with TDR having lower values due to its shallower sensing depth of 15 cm vs. 30 cm. In addition, the soil moisture transect values follow the general contours of the landscape. In particular, site 2 was in a topographic low area that receives large amounts of surface drainage water. Sites 5 and 6 were on hill tops and the surrounding vegetation was noticeably smaller due to gravelly soils and likely greater water stress. Lastly, the second transect in the grassland (sites 7-11) were, in general, drier than the maize survey points (1-6). This is likely due to its higher topographic location and reduced benefits from shading of the maize canopy on the surface.
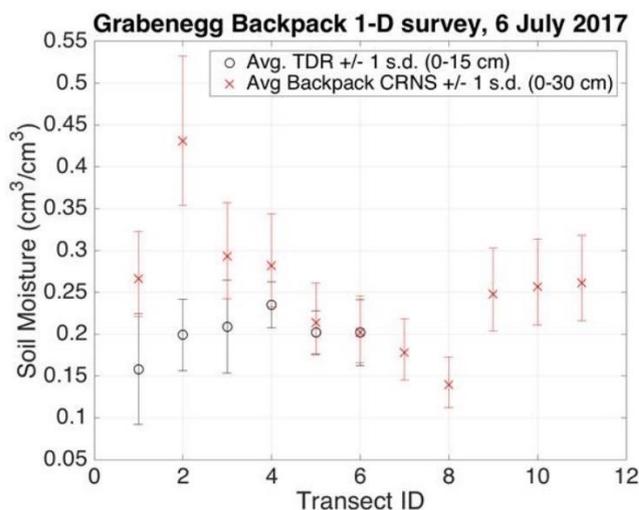


FIG. 10: Results of backpack CRNS and TDR sampling at 11 and six survey points. Note the TDR rods were only 15 cm long and thus resulted in drier observations compared to the backpack, which 'sees' down to ~30 cm. Location 2 is a topographic low area with significant surface drainage

This research was performed under CRPs D1.20.13 on "Landscape Salinity and Water Management for Improving Agricultural Productivity" and D1.50.17 on "Nuclear Techniques for Better Understanding of the Impact of Climate Change on Soil Erosion in Upland Agro-ecosystems".

## Nuclear emergency preparedness in food and agriculture

Member States are increasingly interested in improving the capacity to respond to nuclear emergencies affecting food and agriculture. Lessons learned from the Chernobyl and Fukushima Daiichi Nuclear Power Plant incidents identified critical areas for improvement; this includes data collection (sampling and analysis), data management and data visualization to enable food control and health authorities to respond and disseminate information swiftly to all relevant stakeholders appropriately.

### *Response to nuclear emergencies affecting food and agriculture: sampling, analysis and decision-making*

In 2017, the SWMCNL, through CRP D1.50.15 on 'Response to Nuclear Emergencies Affecting Food and Agriculture', has further developed several protocols and an innovative IT decision support system to facilitate data collection, management and visualization in the response process. The generic, non-country specific approach of the protocols and IT system ensures that all Member States can implement the guidance and tool made available. The development of protocols focuses on large-scale approaches relevant to optimizing the sampling and analytical process, as resources for implementing radioactivity monitoring are limited but critical for swift response.

The concept of Operational Intervention Levels for Soils (OILs for Soils), a sampling optimization tool in the form of pre-determined reference levels of air dose rates linked to radionuclide concentration in soils, has been developed by the SWMCNL team. Continuation of the work includes validation of the concept through comparison of collected data with modelled OILs for Soils values, and testing the feasibility of applying the concept to various emergency scenarios. The work was recognized at the 2017 European Geophysical Union Annual General Assembly (EGU), where the research was selected as an EGU Solicited contribution and highlighted in the programme.

Further, the Decision Support System for Nuclear Emergencies Affecting Food and Agriculture (DSS4NAFA) IT platform is entering the last stages of development before its scheduled beta release in July 2018. DSS4NAFA is an online information system that facilitates agricultural decision making through collection, management, and visualization of data generated during the emergency response and routine monitoring process. An independent review of the system's security setup has been successfully completed by KPMG. Major advancements made include the addition of two modules, the implementation of the system on the IAEA's Azure Cloud, and a pre-release evaluation by two Member States. The two modules: (1) the Advanced Sampling Task Assignment (ASTA) module and (2) the food restriction dashboard module further enhance the robustness of the DSS4NAFA IT decision support system. The ASTA module, jointly developed with the Sustainable Resources Directorate of the Joint Research Centre, the European Commission's science and knowledge service, allows for optimized sampling assignments based on land use maps and crop calendars. The food restriction dashboard suggests response actions based on the decision makers' risk allowance.

Progress has been made on the implementation of DSS4NAFA as a cloud-based system; developers are in discussion with the IAEA's Information Technology Division on the arrangements of the DSS4NAFA testing environment in the IAEA's Azure cloud. Finally, a pre-release system evaluation was performed in partnership with SCK-CEN, the Belgian nuclear research centre, and the Phoenix Leadership Programme of Hiroshima University of Japan. Through this exercise, technical bugs were identified and fixed, and the User Interface and usage flow of the system were improved based on feedback by the evaluators. Several Member States have expressed interest in testing DSS4NAFA upon its release in July 2018.

An animated infographic video is now available demonstrating the principles of DSS4NAFA; for more information see section "Guidelines and Information published in 2017" of this report.

# CAPACITY BUILDING

### *Regional training course on 'Integrated Agricultural Production Systems', 7-11 May 2017, Kuwait Institute of Scientific Research (KISR), Kuwait.*

The SWMCNL supported a regional training course at the Kuwait Institute of Scientific Research (KISR) on the use of nitrogen-15 techniques for enhancing agricultural production systems. This course was carried out under RAS5072 on 'Enhancing the Use of Salt Affected Soils and Saline Water for Crop and Biomass Production and Reducing Land and Water Quality Degradation in ARASIA States Parties'. In total 21 fellows from eight Member States attended the course.

### *Regional training course on 'Use of Advanced Nuclear and Related Tools for Agricultural Water Management and Advanced use of the Crop Simulation Model (AquaCrop)', 3-14 July 2017, Seibersdorf, Austria.*

The SWMCNL also hosted the above training course under RAF5071 on 'Enhancing Crop Nutrition and Soil and Water Management and Technology Transfer in Irrigated Systems for Increased Food Production and Income Generation (AFRA)', attended by 21 fellows from 17 Member States. The training focussed on the use of the cosmic-ray neutron sensor, a nuclear tool used for improving agricultural water management. Further emphasis was put on using AquaCrop, a crop simulation model developed by FAO, for enhancing irrigation scheduling. The course included theory and practical exercises in the field.

# ANALYTICAL SERVICES

In 2017, 4745 plant, soil and water samples were analysed for stable isotopes and 250 samples were measured for fallout radionuclides, respectively, at the SWMCNL. Most analyses (72%) were carried out in support of research and development activities at the SWMCNL focused on the design of affordable isotope and nuclear techniques to improve soil and water management in climate-smart agriculture. An additional analytical focus of the SWMCNL was on $^{13}C$-$CO_2$ and $^{15}N$-$N_2O$ measurements, using the laboratory-based laser isotope analysers.

### *External Quality Assurance: Annual Proficiency Test on $^{15}N$ and $^{13}C$ isotopic abundance in plant materials*

The worldwide comparison of stable $^{15}N$ and $^{13}C$ isotope measurements provides confidence in the analytical performance of stable isotope laboratories and is hence an important tool for external quality control.

The 2017 Proficiency Test (PT) on $^{15}N$ and $^{13}C$ isotopic abundance in plant materials, organized by the University of Wageningen, the Netherlands, and funded by the SWMCNL, has been successfully completed. The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL, wepal.nl) is accredited for the organization of Inter-Laboratory Studies by the Dutch Accreditation Council.

A special evaluation report for FAO/IAEA participants on the analytical performance in stable isotope analysis is issued by the SWMCNL and sent to the participants together with a certificate of participation additionally to the regular WEPAL evaluation report. The participation fee for one round per year is covered by the Joint FAO/IAEA Division.

In total eleven stable isotope laboratories participated in the PT-round 2017: Africa (1): Morocco; Asia (3): Pakistan and Philippines (2 labs); Europe (3): Austria, Belgium and France; Latin America (3): Argentina, Brazil and Chile; and South Pacific (1): New Zealand.

Eight out of nine laboratories participating in the nitrogen analysis reported $^{15}$N-data within the control limits for the enriched plant sample and nine out of nine participating laboratories in carbon analysis reported $^{13}$C isotopic abundance results within the control limits.

# GUIDELINES AND INFORMATION

## *National Geographic Channel explores how nuclear technology helps climate change adaptation*

The work of the Joint FAO/IAEA Division, including that of the SWMCNL, on how nuclear techniques can help the agricultural sector to assess and curtail the worldwide challenge of climate change was shown in December 2016 on the National Geographic Channel in Belgium, France and the Netherlands for a wide audience. Now, the 22-minute documentary is available in English. See: youtu.be/6vIAqqxrzms.

## *Sharing our research progress and connecting with international researchers through the European Geosciences Union General Assembly 2017, Vienna, Austria*

The European Geosciences Union (EGU) 2017 Programme Committee organized the 2017 EGU General Assembly, with 17 500 oral, poster and PICO (i.e. Presenting Interactive COntent™) presentations in 649 scientific sessions. Approximately 14 500 scientists from 107 countries attended this event in Vienna. The SWMCNL activities were reported in 12 presentations (oral, poster and PICO), covering topics on carbon and nitrogen cycling, soil erosion and conservation, quantification of water evapotranspiration and food safety strategies in nuclear emergencies (see list of publications below). The SWMCNL's work on large-scale nuclear emergency response in food and agriculture was selected as a solicited submission and highlighted in the EGU session on 'Geoscience processes related to Fukushima and Chernobyl nuclear accidents'.

## *Proceedings and Outcome Contributions to the Food and Agriculture Organization's Global Symposium on Soil Organic Carbon 2017*

The SWMCNL contributed to proceedings and the final reported outcome of the FAO's 2017 Global Symposium on Soil Organic Carbon (SOC). This symposium highlighted the importance of SOC in climate change adaptation and mitigation, food security, soil stability and sustainability. It provided a platform for efforts measuring, monitoring, mapping, maintaining and increasing SOC in natural and agricultural ecosystems from around the world to be shared in oral and poster presentations. A summary of the symposium and concluding recommendations were compiled into a final FAO document, "Unlocking the potential of Soil Organic Carbon," and can be found here: fao.org/documents/card/en/c/25eaf720-94e4-4f53-8f50-cdfc2487e1f8/.

## *European Development Days*

The SWMCN Subprogramme attended the 2017 European Development Days (EDD) (eudevdays.eu/) as part of efforts to showcase how science is supporting development. The work in Benin on the pulses and in Sudan on small-scale irrigation were highlighted. The EDD are Europe's leading forum on development and international cooperation. Organised by the European Commission, the forum brings the development community together each year to share ideas and experiences in ways that inspire new partnerships and innovative solutions to the world's most pressing challenges.

## *Contribution to the IAEA Nuclear Technology Review 2017*

The SWMCNL contributed to the IAEA's Nuclear Technology Review 2017 (see pp. 32-34) by reporting on research and development progress in the field of nuclear and related techniques for improving agricultural water management. Agriculture accounts for approximately two thirds of global freshwater consumption. To achieve the yields required for rising populations, agricultural systems must strive for efficiency. The cosmic ray neutron sensor is a novel device that can capture and

quantify soil water content over large areas without the time-consuming and invasive aspects of traditional assessment systems. The soil moisture information provided by this device is leading to better management of increasingly scarce water resources. For more information, see: iaea.org/About/Policy/GC/GC61/GC61InfDocuments/English/gc61inf-4_en.pdf

## World Soil Day: Caring for the Planet Starts from the Ground and Nuclear Techniques Can Help

Nuclear science provides ways to understand soil health and generate data to help improve its quality for food production, make it more resilient to climate change and protect it for the future. On the 2017 World Soil Day, examples of the impacts of nuclear science for soil conservation in regions around the world were presented to underscore the message of this year's World Soil Day theme: 'Caring for the planet starts from the ground'. The full stories from Viet Nam, Morocco and Chile are available at iaea.org/newscenter/news/world-soil-daycaring-for-the-planet-starts-from-the-ground-and-nucleartechniques-can-help.

## DSS4NAFA – a new animated infographic video by the Joint FAO/IAEA Division

A new animated infographic video on "DSS4NAFA" provides the lay audience with a comprehensible introduction to the Decision Support System for Nuclear Emergencies Affecting Food and Agriculture, which has been developed by the Joint FAO/IAEA Division. Immediately following an incident, DSS4NAFA downloads and analyses data on radiation contamination rates in the area and, within minutes, gives decision makers a range of precautionary options for protecting food and agriculture. It computes what additional data is needed to inform decisions that will keep the population safe. It combines land-use maps and crop calendars with sampling protocols for each crop and vegetation in potentially affected areas and instantly creates detailed assignments for sample collection teams. The samples are analysed in laboratories linked to DSS4NAFA, and results recorded directly into the decision support system. Using innovative computer algorithms and geo-visualization platforms the continuously evolving situation is visible at a glance, enabling decision makers to formulate up-to-the-minute guidance. This video, and many others, can also be found at naweb.iaea.org/nafa/resources-nafa/multimedia.html, where they available in Arabic, Chinese, English, French and Spanish.

## New FAO publication: Use of $^{137}$Cs for soil erosion assessment

This publication provides guidance on the use of $^{137}$Cs for soil erosion assessment. It covers major components of the $^{137}$Cs method, starting from its principles, the sampling strategy, gamma spectroscopic measurements, estimation of erosion rates with conversion models, data interpretation and erosion model validation. The publication is targeted at a wide audience, such as researchers, agricultural and environmental experts, decision makers, farmers and students and can be downloaded at fao.org/documents/card/en/c/74f5f529-4cc5-472b-9ed1-b29158d24ff5.

## New open-access FAO/IAEA publication: Cosmic Ray Neutron Sensing: Estimation of Agricultural Crop Biomass Water Equivalent, published by Springer

This open access book provides methods for the estimation of Biomass Water Equivalent (BEW), an essential step in improving the accuracy of area-wide soil moisture by cosmic-ray neutron sensors (CRNS). Three techniques are explained in detail: (i) traditional *in-situ* destructive sampling, (ii) satellite based remote sensing of plant surfaces and (iii) biomass estimation via the use of the CRNS itself. The advantages and disadvantages of each method are discussed along with step by step instructions on proper procedures and implementation. The publication can be downloaded at springer.com/br/book/9783319695389.

## SWMCN success stories in 2017

Fifteen success stories were published by the SWMCN Subprogramme in 2017 highlighting examples of country impacts derived through the use of nuclear and nuclear-related techniques in the

improvement of soil and water management and crop nutrition across Africa, Asia and Latin America. Two stories were prepared with the support of the SWMCNL, i.e. (i) Caring for the Planet Starts from the Ground and Nuclear Techniques Can Help and (ii) Area-wide Measurements of Soil Water Improve Management of Scarce Water Resources in Agriculture. These stories can be downloaded at https://www.iaea.org/news?year%5Bvalue%5D%5Byear%5D=2017&type=All&topics=3050&keywords=&=Search.

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# AN UPDATE ON THE ReNuAL PROJECT:
# THE FAO/IAEA AGRICULTURE & BIOTECHNOLOGY LABORATORIES

ReNuAL is the initiative to modernize the eight aging laboratories in Seibersdorf, Austria that are managed by the IAEA's Department of Nuclear Science and Applications. These laboratories, five of which belong to the Joint FAO/IAEA Division, strengthen Member States' capacities to use nuclear and related techniques in food and agriculture, human health, the environment and scientific instrumentation.

## Inauguration of the new Insect Pest Control Laboratory

It only took 15 months to transform an empty site of dirt into a new building, surrounded by grass and trees. On 25 September 2017, IAEA Director General Yukiya Amano inaugurated the new Insect Pest Control Laboratory. Representatives of more than 35 Member States joined the celebration in Seibersdorf and toured the new laboratory. With over 1700 m$^2$ of laboratory space, the new facility will substantially increase the ability of the Joint FAO/IAEA Division to assist Member States in controlling harmful insect pests. The transition from the current IPCL to the new IPCL will begin as planned in the first quarter of 2018.

"With new and modern facilities, the Insect Pest Control Laboratory will in future be able to do even more to help Member States control insect pests that endanger our crops, our livestock and our health" said Director General Amano during the event. He was joined in delivering remarks by Indonesian Ambassador and Chair of the IAEA Board of Governors HE Darmansjah Djumala, Austrian Ambassador HE Christine Stix-Hackl, German Ambassador and Co-chair of the Friends of ReNuAL HE Friedrich Däuble, South African Ambassador and Co-chair of the Friends of ReNuAL HE Tebogo Joseph Seokolo and Assistant Director General Ren Wang of the Food and Agriculture Organization of the United Nations.



*The IPCL building close to completion*

## Flexible Modular Laboratory construction fully funded

The IAEA will now be able to complete construction of the second new laboratory facility, the Flexible Modular Laboratory (FML), thanks to recent extra-budgetary contributions from Member States. Since May 2017, €5.7 million have been raised for ReNuAL+ construction to achieve full funding for the

Animal Production and Health Laboratory, the third planned laboratory of the FML. Construction of the first two laboratories is fully funded under ReNuAL. In total, the IAEA has raised over €32 million in extra-budgetary funds plus in-kind contributions for ReNuAL and ReNuAL+ from 31 Member States and other donors. FML construction began in April 2017 and is planned for completion at the end of 2018.

Donors were recognized at an event during the IAEA's General Conference that featured the unveiling of the ReNuAL/ReNuAL+ donor wall. Director General Amano delivered remarks along with representatives of the Co-chairs of the Friends of ReNuAL: Ms Thembisile C. Majola, Deputy Minister of Energy for South Africa, and Mr Thorsten Herdan, Director General for Energy Policy of the Federal Ministry for Economic Affairs and Energy of Germany. The donor wall will be permanently displayed in the new Insect Pest Control Laboratory in Seibersdorf.

The upcoming focus for resource mobilization is now on outfitting the laboratories through procurement and partnerships as well as enhancement of the laboratories remaining in the current buildings.



*Perspective of the FML building currently under construction*

## Equipment manufacturer donates to support food safety

Shimadzu Corporation, a commercial company, has come forward to support the enhancement of the laboratories in Seibersdorf: The Food and Environmental Protection Laboratory of the Joint FAO/IAEA Division will be able to increase its efforts to help Member States test for contaminants in food thanks to an in-kind donation of sophisticated detection equipment. Director General Amano signed the agreement for the donation with Shimadzu Chairman Mr Akira Nakamoto in Tokyo on 2 October. The Food and Environmental Protection Laboratory will use the new machine to train scientists from all over the world in applying state-of-the-art analytical methods to test for contaminants, such as pesticides and veterinary drug residues, in basic food products. It will also support FAO/IAEA research on reliable methods to confirm the origin of food and to test for food adulteration. The donated machine is a liquid chromatograph with triple quadrupole mass spectrometric capabilities (LC-MS/MS).